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## Detection of *Adenovirus* antigen and its correlation with clinical signs among children with gastroenteritis

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### Abstract

A total of 90 stool sample was collected from patients with gastroenteritis to detect adenovirus antigen among diarrhea cases. They were tested by general stool examination (GSE), rapid immunochromatographic test and Enzyme Linked Immunosorbent Assay (ELISA). GSE showed that adenovirus gastroenteritis infection resulted in non-bloody diarrhea, the existence of RBCs in 7% and Pus in 37% of the samples, *Entamoeba histolytica* trophozoite and cyst were seen in 3% and 2% of the samples respectively. The rapid test showed that 21% of samples were positive for rotavirus, 8% for adenovirus and 3% for astrovirus. Meanwhile, the ELISA test showed that adenovirus was positive in 9% of the samples. These findings established the evidence that adenovirus is significantly the second viral agent that cause gastroenteritis among children after rotavirus. Furthermore, it must be stated that rapid diagnostic methods are important for detection of adenovirus infection.

**Keywords:** *Adenovirus* antigen, Gastroenteritis, Rapid test and ELISA.

## التحري عن مستضد الفيروس الغدي وعلاقته مع وجود علامات سريرية بين الأطفال الذين يعانون من التهاب المعدة والأمعاء

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### الخلاصة

تم جمع ما مجموعه 90 عينة براز من المرضى الذين يعانون من التهاب المعدة والأمعاء للتحري عن مستضد الفيروس الغدي بين حالات الإسهال. تم اختبارها بواسطة فحص البراز العام، فحص الترجيل الكروماتوغرافي المناعي السريع وفحص الامتزاز المناعي المرتبط بالانزيم. أظهر فحص البراز العام أن الإصابة بالتهاب المعدة والأمعاء للفيروس الغدي أسفرت عن الإسهال غير الدموي، وجود كرات الدم الحمراء في 7% والقيح في 37% من العينات، الطور المغتذي والطور الكيسي لطفيلي المتحولة الزحارية شوهد في 3% و 2% من العينات على التوالي. أظهر الفحص السريع أن 21% من العينات كانت إيجابية للفيروس الدوار، و 8% للفيروس الغدي و 3% للفيروس النجمي. في الوقت نفسه أظهر فحص الامتزاز المناعي المرتبط بالانزيم أن الفيروس الغدي كان ايجابي في 9% من العينات. انشأت هذه النتائج الأدلة على ان الفيروس الغدي هو ثاني

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العوامل الفيروسية التي تسبب التهاب المعدة والأمعاء وبشكل ملحوظ لدى الأطفال بعد الفيروس النوار. علاوة على ذلك، لا بد من ذكر أن طرق التشخيص السريع مهمة للتحرر عن الإصابة بالفيروس الغدي.

### Introduction

Viral gastroenteritis is a global problem in infants and young children [1]. Symptoms of the disease are severe diarrhea, vomiting, fever, and rapid dehydration [2]. Diarrhea remains the second leading cause of death around the world for children under five years of age [3]. After Rotavirus infection, Adenovirus is the second cause of severe and acute gastroenteritis in children under five years of age [4]. Human adenoviruses are non-enveloped, icosahedral with linear dsDNA genome. They are grouped in seven species (Human adenovirus A to G) with 57 accepted types [5]. Group F of adenovirus including types 40 and 41 are the main etiologies of 1-20% of acute gastroenteritis and 50% of all adenoviruses found in stool samples are types 40 and 41 [6]. The ability of adenoviruses to cause acute infection in both the gastrointestinal tract and the respiratory system, makes them quite important. It has been reported that they cause about 5% of all infectious diseases in infants and nearly 3% in children from 2-4 years old [7]. Epidemiological studies in different regions of the world have shown that adenovirus infection occurs throughout the year with increased incidence during winter [8].

The incidence of enteric adenovirus infections in childhood is reported to be between 3.1% to 13.5% in studies performed in Europe, Asia, Northern and Southern America [9].

Regarding the Iraqi children, diarrhea is a major cause of illness and death. Thus, many reports indicated rotavirus infection, but little data is recorded about adenovirus in our population, so this project was undertaken to investigate the prevalence of this virus and its role in viral diarrhea.

### Materials and Methods

**Samples:** stool samples were collected from 90 children with ages ranging from less than one to 15 years admitted to the central teaching hospital of pediatric with symptoms of acute gastroenteritis between November 2013 and April 2014. A total of 45 stool samples from apparently normal children, which were both age and gender matched, were used as control group. Each stool sample was taken once from these children in clean containers according to routine procedures and the instructions of the physician, divided into two parts, and stored undiluted at -20°C until the time of analysis. The 90 children admitted to the hospital and diagnosed with acute gastroenteritis, were 63(70%) males and 27(30%) females as shown in table -1.

**Table 1-** Distribution of samples according to age and gender.

Samples	Age groups (years)	No.	Males	Females
Patients	<1	28	21	7
	1-6	50	34	16
	>6-15	12	8	4
	Total	90	63	27
Control group	<1-15	45	26	19

**General stool examination:** Fresh samples were tested Macroscopically (visual observation of the sample) and Microscopically as in the routine work: a drop of saline was placed on a clean slide and a small amount of stool was mixed with it, covered with cover slip, then examined under 10X and 40X objectives by light microscope (Leitz optical microscope, Germany)[10-11].

**Rapid immunochromatographic test:** samples were tested for detection of Adenovirus antigen by specific kit (Cer Test Biotec, Spain) [12]. This one step combo card test is a coloured chromatographic immunoassay for the simultaneous qualitative detection of adenovirus antigen in stool samples. This test was carried out according to the procedure mentioned by the manufacturer.

**ELISA test:** samples were tested by an ELISA kit specific for adenovirus antigen [13]. Sandwich enzyme linked immunosorbent assay for qualitative detection of Adenovirus antigen was carried out by Adenovirus Fecal ELISA kit (Diagnostic Automation, INC., USA), according to the manufacturer's instructions. Absorbance were read by microplate reader (BioTek instruments, USA) at 450-630nm and the cut-off was 0.15 and above.

**Statistical Analysis:** Calculation of the mean values and standard deviations were made for the optical density (O.D.) results of ELISA test. The statistical significance of difference between positive and

negative samples was assessed by t-test and probability value of  $P < 0.05$  was considered statistically significant [14].

### Results and Discussion

The macroscopic examination of the stool samples showed that adenovirus gastroenteritis infection resulted in diarrhea with a watery or loose stool consistency, in which all the positive samples tested were watery or loose stools. There was no blood in the stool samples of adenovirus infection, this may indicate that adenovirus results in non-bloody diarrhea and these results are agreed with the fact that if the stool is bloody, the cause is less likely to be viral and more likely to be bacterial [15-16]. The microscopic examination of the stool samples showed the existence of RBCs in 7% and Pus in 37% of the samples. *Entamoeba histolytica* trophozoite and cyst were seen in 3% and 2% of the samples respectively (Table -2). About 33% of stool samples that had RBCs and 15% of those containing pus were associated with *E. histolytica*.

**Table 2-** Observations of stool microscopic examination among the gastroenteritis patients and control group samples in relation to adenovirus positive cases as detected by ELISA.

Samples	No.	RBCs presence (%)	Pus cell presence (%)	<i>Entamoeba histolytica</i>	
				Trophozoite (%)	Cyst (%)
Adenovirus positive	8	2 (25)	6 (75)	1 (12.5)	1 (12.5)
Total patients	90	6 (7)	33 (37)	3 (3)	2 (2)
Control group	45	-	-	-	-

Microscopic stool examination showed that 25% of adenovirus positive samples containing RBCs had coexistence with *E. histolytica*, but were not from only adenovirus infection, 75% of all adenovirus positive samples were containing pus and 25% of adenovirus positive samples were showing co-existence with *E. histolytica*. These results showed that most of the existing RBCs and pus (inflammatory factors) in adenovirus positive samples were caused by parasitic infections by *E. histolytica*, which mostly causes amebic dysentery. This confirmed the fact that adenovirus is one of the reasons of non-inflammatory diarrhea and these factors can be interpreted that there were other intestinal pathogens [17].

Out of the 90 stool samples tested by rapid test, 29 samples (32%) were positive for rotavirus, adenovirus and astrovirus. The percentage of Rotavirus infections were the highest compared to adenovirus and astrovirus, with percentages 21%, 8% and 3% respectively, however, rotavirus and adenovirus were detected among control group with percentages 7% and 2% respectively, as shown in table -3.

**Table 3-** Results of rapid test for adenovirus, rotavirus and astrovirus antigens detection in stool samples of the gastroenteritis patients and control group.

Samples	Total No.	No. of virus positive (%)		
		Rotavirus	Adenovirus	Astrovirus
Patients	90	19 (21)	7 (8)	3 (3)
Control group	45	3 (7)	1 (2)	-

The viral etiology of gastroenteritis were detected in percentages to be close to the results of some other studies done on viral gastroenteritis in children in Iraq and other countries for detection of viruses in stool samples by rapid test. In previous studies done in Iraq, rotavirus infections were higher than adenovirus in infants and young children, the percentages of rotavirus among diarrhea cases were recorded between 16.9% and 91.3%, while adenovirus percentages were between 2.6% and 20.4% [1, 6, 18-19]. These results were similar to the results of some studies in Turkey [9-20], who used rapid test for detection of rotavirus and adenovirus antigens in stool samples, and rotavirus was detected with percentages between 9.4% and 25%, while adenovirus percentages was between 1.9% and 8.6% in infants and children with age up to 14 years. In one study in Turkey, rotavirus and adenovirus were detected by rapid test in the same percentage 14% in infants and children up to 12 years of age with acute gastroenteritis [21]. Only exception in the study that has been carried out in Guatemala in which adenovirus cases were three fold more than the cases of rotavirus, when these two viruses were detected in stool samples by ELISA test, and this situation was attributed to the climatic differences

[22]. Astrovirus when compared to other studies done previously, its percentage was in accordance with the reported worldwide, it is less common than rotavirus and adenovirus [23], however, there is no previous recorded data on astrovirus as an etiologic agent of viral gastroenteritis in Iraq. The presence of rotavirus and adenovirus in samples of control group was agreed with the results of a study in Brazil [24], in which the two antigens were detected in 10.1% and 1.7% of control group stools, respectively by ELISA. This might indicate that the viruses may cause subclinical infections that infect an apparent normal individuals [25].

All samples including positive samples by rapid test were subjected to ELISA, the positivity of these samples was confirmed and another sample turned positive from the remaining negatives, the sample in control group that was positive in rapid test, by ELISA, it revealed to be false positive. This may be due to the higher sensitivity and specificity of ELISA test than rapid test. So altogether, the detection of adenovirus based on ELISA was 9% as shown in table -4.

**Table 4-** Results of ELISA test for adenovirus antigen detection in gastroenteritis patients and control group samples.

Samples	No.	O.D Mean $\pm$ S.D
Patients positive	8	(0.35 $\pm$ 0.17)*
Patients negative	82	(0.09 $\pm$ 0.03)
Total patients	90	-
Control group	45	(0.07 $\pm$ 0.015)
P- value	P<0.05	*S
		*Cut-off $\geq$ 0.15

\*S = Significant

\*There was significant difference between the positive and negative samples as compared to the control group.

These results were close to the results of an Iraqi study for determination of the prevalence of viral etiology of diarrhea in children less than 5 years of age in Baghdad, in which adenovirus was detected by using direct ELISA test for antigen detection and a prevalence of 6% was indicated [26]. Also it was agreed with other study in Iran, which showed close percentage of adenovirus 7.8% among children with ages up to 12 years old using ELISA for detection of adenovirus antigens in stool samples [27]. On the other hand, the presented data disagreed with number of studies were carried out in Iran by ELSIA tests for adenovirus antigen detection, in which adenovirus prevalence rates were ranging between 2.6% and 3.3% among children with age up to 5 years [28-29], while other studies showed adenovirus prevalence between 2.3% and 5.1% among children with age less than 10 years and 7 years respectively [7-8]. The differences between the presented results and results of the other studies may be due to the age, immunity, nutrition status of the patients, season and geographic region [2]. In this research, accordingly to our data adenovirus is significantly the second viral agent that cause gastroenteritis among children after rotavirus. Adenovirus antigen can be detected routinely in stool samples among children, especially in autumn and winter using rapid test or ELISA as they show similar results. However we suggest the use of rapid test in diagnosis since it is easy to use and lower in cost.

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