

# Study The Cellular, Humoral Immune Response And The Prevalence Of Enteric Adenovirus, Rotavirus and Norovirus Causing Diarrhea In Infants

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## Abstract :

In this research many different viruses that may cause gastroenteritis had been studied, including Rotaviruses, Noroviruses, Adenoviruses, type 40 or 41, Sapoviruses, and Astroviruses. The main symptoms of viral gastroenteritis are watery diarrhea and vomiting

In this study we used Rapid chromatography immunoassay for the qualitative detection of Rotavirus and Adenovirus in human feces specimens , enzyme sorbent immuno assay technique for detection Norovirus. this study included 100 blood and feces specimens. (80) for infected patient that lies in (16) specimens was acute infantile diarrhea with Norovirus infection and 11 specimens was Acute infantile diarrhea with adenovirus infection , (20) specimens was Rotavirus, (33) specimens was Acute infantile diarrhea without Norovirus, Adenovirus and Rotavirus infection and (20) specimens as a control group . Distribution of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group according to the type of feeding. In the breast feeding were 37.5%, 18.18%,35%,45.45%,50% respectively but it were 62.5%, 81.82%,65%,54.55%, 50 % in Mixed feeding respectively. The statical analysis was high significant  $P<0.05$  in Mixed feeding in comparing with Breast feeding. The study shows that the infant (7-12)month have high percentage of infected with virus . The level of non specific immune response by using differential leukocytes counts [DLC] was studied also. The statical analysis of percentage of Neutrophils, Lymphocytes and Monocytes was lower significant in Acute infantile diarrhea with Norovirus, Adenovirus and Rotavirus and Acute infantile diarrhea in comparing with control group. and high significant  $P<0.05$  the average level of immunoglobulin G [IgG]of Acute infantile diarrhea comparing with control group .

## الخلاصة :

في هذا البحث تم دراسة عدة انواع من الفايروسات التي يمكن أن تسبب التهابا معويا في القناة الهضمية منها فيروس الروتا والادينو والرونو والنوع ٤٠ و ٤١ فايروس السابرو والاسستروفيروس . ان الأعراض الرئيسية في الالتهاب المعوي هو القيء والإسهال المائي . حيث تم في هذه الدراسة استخدام تقنية الترحيل الكروماتوكرافي السريع للكشف عن فيروس الروتا والادينو فايروس في عينات الخروج . وتقنية الاليزا للكشف عن النوروفيروس . وقد تضمنت هذه الدراسة ١٠٠ عينة من الدم والخروج، ٨٠ عينة مرضى الإسهال كان منها ١٦ عينة مرضى الإسهال الحاد مصابين بالنوروفيروس و ١١ عينة مرضى الإسهال الحاد مصابين بالادينو فايروس و ٢٠ مرضى الإسهال الحاد مصابين بالروتافيروس و ٣٣ عينة مرضى الإسهال الحاد غير مصابين بالنورو والادينو والروتا فايروس و ٢٠ عينة أصحاء (مجموعة سيطرة). وقد اثبت التحليل الإحصائي لنسب انتشار الفيروسات النورو والادينو والروتافيروس ومجموعة السيطرة وجود فروقات معنوية عند مستوى الاحتمال ٠.٠٥ بين نوعي التغذية حليب الأم والرضاعة المختلطة ففي حالة حليب الام كانت النسب ٣٧.٥%، ١٨.١٨%، ٣٥.٤٥%، ٥٠.٤٥% على التوالي في حين كانت في الرضاعة المختلطة ٦٢.٥%، ٨١.٨٢%، ٦٥.٥٥%، ٥٠.٥٠% على التوالي. وان الفئة العمرية (٧-١٢) شهر سجلت أكثر نسبة انتشار للفيروسات. كما تضمنت الدراسة حساب مستوى الاستجابة المناعية الخلوية غير المتخصصة وقد اثبت التحليل الإحصائي انخفاضا معنويا عند مستوى احتمال ٠.٠٥ واضحا في مستوى كريات الدم البيض في عينات دم مرضى الإسهال المصابين بالفيروسات مقارنة بمجموعة السيطرة . وارتقا معنويا ملحوظا في مستوى الاستجابة المناعية الخلوية المتخصصة [IgG] في مصل دم المرضى مقارنة بمجموعة السيطرة.

## Introduction:

Inflammation of the gastrointestinal tract, involving both the stomach and the small intestine and resulting in acute diarrhea (Middleton and Petric ,1977) The inflammation is caused most often by an infection from certain viruses or less often

by bacteria, their toxins, parasites, or an adverse reaction to something in the diet or medication (Madeley *et al.*, 1977; Madeley, 1986)). Different species of bacteria can cause gastroenteritis, including *Salmonella*, *Shigella*, *Staphylococcus*, *Campylobacter jejuni*, *Clostridium*, *Escherichia coli*, *Yersinia*, and others. some of cases of gastroenteritis as food borne illness are due to norovirus, and the majority of severe cases in children, are due to rotavirus. Other significant viral agents include adenovirus and astrovirus (Uhnnoo *et al.* , 1984).

Acute infection with norovirus results in reversible histopathologic lesions in the jejunum but not the stomach or rectum and manifests with vomiting and diarrhea. Changes appear within 24 hours of viral challenge, remain through the height of the illness, and persist for a variable time after the illness (Joncas and Pavilanis , 1996). Intestinal villa appear blunted, but the mucosa remains intact. On electron microscopy, epithelial cells are intact, but microvilli are shortened and have widened intercellular spaces (Hyman and Ament ,1982).

The major mode of transmission is fecal-oral spread, usually through consumption of a fecally contaminated vehicle (either food or water) Because the infectious dose is as low as 100 viral particles, low-level contamination of food and water can lead to outbreaks (Spencer and Cherry ,1981; Adams, *et al.* ,1984)). A food vehicle may be fecally contaminated at its source (such as oysters harvested from contaminated waters or fruits and vegetables grown in contaminated environments (Ruuskanen, *et al.*, 1985) Transmission of noroviruses occurs year-round, but higher disease incidences occur in the winter months in temperate climates (Yolken, *et al.*,2000).

Rotavirus is a genus of double-stranded RNA virus in the family Reoviridae (Kidd, *et al.*,1985;Brandt, *et al.*,1999 ;Drachman ,2004;). It is the leading single cause of severe diarrhoea among infants and young children There are seven species of this virus, referred to as A, B, C, D, E, F and G. Rotavirus A, the most common, causes more than 90% of infections in humans (Van ,*et al.*,1992). Rotavirus is transmitted by the faecal-oral route (Lew *et al.*,1991). It infects cells that line the small intestine and produces an enterotoxin, which induces gastroenteritis, leading to severe diarrhoea and sometimes death through dehydration (Brandt, *et al.*,1985). More than 500,000 children under five years of age die from rotavirus infection each year and almost two million more become severely ill. Rotavirus infections rarely cause other complications and for a well managed child the prognosis is excellent .There are rare reports of complications involving the central nervous system (CNS) where rotavirus was detected in the fluid of the (CNS) in cases of encephalitis and meningitis (Romano, *et al.*,2000).

Adenoviruses are medium-sized (90–100 nm), non enveloped (naked) icosahedral viruses composed of a nucleocapsid and a double-stranded linear DNA genome (Bartlett, *et al.*,2005 ;Welliver, *et al.*,2008) . There are over 52 different serotypes in humans. Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water (Bishop, *et al.*,1973). Adenoviruses are primarily spread via respiratory droplets, however they can also be spread by fecal routes (Gaudini, *et al.* ,1973).

## Materials and Methods :

Collection of the specimens : this study included :

- 1- collection 80 stool and blood specimens from infants with acute diarrhea in Babylon maternity and children hospital .
- 2- collection 20 stool and blood specimens from healthy infants ( as a control group)

### Laboratory tests :

The laboratory diagnostic tests on the collected stool specimens included :

Diagnosis of Human adenoviruses and rotavirus infection :

Rapid chromatography immunoassay for the qualitative detection of adenoviruses and rotavirus in human feces specimens in this test the membrane is pre-coated with anti-adenoviruses antibody on the A test line region and with anti- rotaviruses antibody on the R test line region . during testing the specimen react with the particle coated with anti- adenoviruses antibody . the mixture migrate upward on the membrane chromatographically by capillary action to react with anti- adenoviruses antibody and generate blue line . the presence of the colored line in test line region indicates a positive results , while their absence indicates a negative result .

Differential Leukocytes Counts [DLC] :-

-Placed one drop of blood specimen on the glass slide . and spread this blood drop by using other glass slide to form a blood film and leave it for drying , add lishman stain to the slide for 2 min , then wash the slide by using D.W. and leave it 5min for dry then examined by using oil immersion . count 100 leukocytes and account the percentage of each W.B.C.s.

Measurement concentration of immunoglobulin IgG by using Radial immunodiffusion assay :-

-Add 5µl from serum specimen in well of plate (12 wells in each plate) by using micropipette , then leave it to settle for 15-20min , and covered these plates and keep it for 72 hours ,then measurement the precipitation ring around each well that result from reaction between antibody and antigen was measured using immuno viewer .

### Statistical analysis :

Statistical analysis were conducted to describe different variables and parameters in the research, and to describe relationship with each other as well. Calculation of mean value and standard deviation (SD) were made for immunological parameters.

The statistical significance of difference in mean of variable between more than two groups was assessed by ANOVA test .Probability values of  $P < 0.05$  were considered statistically significant.

### Results and Discussion:

Our study included prevalence of infected infants with Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group according to the Type of feeding, Age, the level of non-specific immune response and study the level of Humoral immune response [IgG]. Distribution of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group according to the Age [1-6] months was (12.5%,18.18% ,15%,25%) respectively and it was (56.25%, 63.64% ,50% , 25%) in [7-12] months respectively. but it was (25.25%, 63.64% ,50% , 25%,18.18%,30%,36.37%,25% ) in [13-24]months respectively . our study revealed that Norovirus, Adenovirus , Rotavirus infection was common in infants between[7-12] months [table 1] .This results are nearly the same as that proved ( Welliver, *etal.*,2008) who found that the range of infants age was under 12 months . (Bishop, *etal.*, 1973)found a significant difference in the numbers of Adenovirus isolated from infants with diarrhea and from a control group; Adenovirus was recovered from 17% of infants with diarrhea but from only 5% of normal infants of the same age . Agents of viral gastroenteritis such as Astrovirus, Rotavirus, Norovirus ,and Adenovirus are

common pediatric pathogens accounting for many physician visits, hospital admissions, and nosocomial infections (Gaudini *et al.*,1973).

Table (1): Distribution of Acute infantile diarrhea with Norovirus, Adenovirus and Rotavirus infection according to the Age (months) .

Groups		Age (months)				total
		1-6	7-12	13-24	25-60	
Acute infantile diarrhea with Norovirus infection	NO.	2	9	4	1	16
	%	12.5	56.25	25	6.25	100
Acute infantile diarrhea with Enteric Adenovirus infection	NO.	2	7	2	0	11
	%	18.18	63.64	18.18		100
Acute infantile diarrhea with Rotavirus infection	NO.	3	10	6	1	20
	%	15	50	30	5	100
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	NO.	6	6	12	9	33
	%	18.18	18.18	36.37	27.27	100
Control	NO.	5	5	5	5	20
	%	25	25	25		100
Total	NO.	18	37	29	16	100
	%	18	37	29	16	100

The prevalence of infected infants with Norovirus, Adenovirus , Rotavirus and control group according to the type of feeding it was 37.5% ,18.18% 35%and 50% respectively in breast feeding .but it was 62.5% ,81.82% 65%and 50% respectively in mixed feeding[table 2]. We have previously shown that Astrovirus ,Adenovirus and Rotavirus are frequently present in stools of children with symptomatic gastroenteritis in our hospital (Hess, 2000). Our study included accountance the percentage of neutrophils in blood specimens of acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group ( $55.060 \pm .5657$  ,  $51.155 \pm 1.209$  ,  $50.9400 \pm 1.3294$  and  $57.4950 \pm 0.700$ ) respectively [table 3]. the statistical analysis in freedom degree ( 2,3 ) was significant  $P < 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection in comparing with control group . but it was no significant  $P > 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection in comparing with acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection .

Table (2): Distribution of Acute infantile diarrhea with Norovirus, Adenovirus and Rotavirus infection according to the Type of feeding .

Groups		Type of feeding		total
		Brest	Mixed	
Acute infantile diarrhea with Norovirus infection	NO.	6	10	16
	%	37.5	62.5	100
Acute infantile diarrhea with Enteric Adenovirus infection	NO.	2	9	11
	%	18.18	81.82	100
Acute infantile diarrhea with Rotavirus infection	NO.	7	13	20
	%	35	65	100
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	NO.	15	18	33
	%	45.45	54.55	100
Control	NO.	10	10	20
	%	50	50	100
Total	NO.	40	60	100
	%	40	60	100

Table(3):the percentage of Neutrophils % in blood specimens of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection and control group .

Groups	NO.	Mean	S.D	S.E
Acute infantile diarrhea with Norovirus infection	16	55.0600	.5657	.4000
Acute infantile diarrhea with Enteric Adenovirus infection	11	51.1550	1.2092	.8550
Acute infantile diarrhea with Rotavirus infection	20	50.9400	1.3294	.9400
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	33	54.0250	1.3223	.9350
Control	20	57.4950	.7000	.4950
Total	100	53.7350	2.7219	.8607

[Table 4] appeared the percentage of lymphocytes in blood specimens of acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group was ( $26.145 \pm 0.445$ ,  $25.035 \pm 9.192$ ,  $23.385 \pm 0.544$  and  $28.795 \pm 0.841$  ) respectively. the statistical analysis in freedom degree ( 2,3 ) was significant  $P < 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection in comparing with control group . but it was no significant  $P > 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection in comparing with acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection .

[Table 5] appeared the percentage of monocytes in blood specimens of acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group was ( $7.39 \pm 0.55$ ,  $6.51 \pm 0.55$ ,  $6.10 \pm 0.17$  and  $9.33 \pm 0.47$  ) respectively. the statistical analysis in freedom degree ( 2,3 ) was significant  $P < 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection in comparing with control group . but it was no significant  $P > 0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection in comparing with acute infantile diarrhea without Norovirus, Adenovirus , Rotavirus infection . low levels of differential leukocytes may be due to found or replication the viral nucleic acid within host cells.

Table (4): the percentage of lymphocytes % in blood specimens of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection and control group .

Groups	NO.	Mean	S.D	S.E.
Acute infantile diarrhea with Norovirus infection	16	26.1450	.4455	.3150
Acute infantile diarrhea with Enteric Adenovirus infection	11	25.0350	9.192E-02	6.500E-02
Acute infantile diarrhea with Rotavirus infection	20	23.3850	.5445	.3850
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	33	27.4850	.6859	.4850
Control	20	28.7950	.8415	.5950
Total	100	26.1690	2.0279	.6413

Table (5): the percentage of Monocytes % in blood specimens of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection and control group .

Groups	NO.	Mean	S. D	S. E
Acute infantile diarrhea with Norovirus infection	16	7.3950	.5586	.3950
Acute infantile diarrhea with Enteric Adenovirus infection	11	6.5100	.5515	.3900
Acute infantile diarrhea with Rotavirus infection	20	6.1050	.1768	.1250
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	33	8.8450	.2475	.1750
Control	20	9.3350	.4738	.3350
Total	100	7.6380	1.3731	.4342

Also our study included account the average level of immunoglobulin G [IgG] of acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus and control group was (2325.9500±19.0500, 1310.500±10.00, 2318.0500± 43.0500, and 246.8700± 23.4300) respectively [Table 6] . The statistical analysis was high significant  $P<0.05$  in acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus in comparing with control group .

Several studies (Little,1996; Hess, 2000) have been reported on the conjunctivitis and gastroenteritis immunoglobulin level in normal subjects, but not much information is available on the serum immunoglobulin levels in acute viral gastroenteritis.

Our results agree with (Hess, 2000) findings high level of immunoglobulin in serum acute viral gastroenteritis. (Unicomb *et al.*,1998) studied some patients with gastroenteritis diseases and found only the IgA level rising in the stool specimens. few studies are available on the serum immunoglobulin levels in viral infections. (Grohmann *et al.*,1993) significantly raised serum IgG, IgA, and IgM levels. In another study no significant alteration was noted in the serum IgA level in acute adenovirus gastroenteritis, while there was a significant fall in the serum IgG and IgM levels.

Table (6): Average level of immunoglobulin G [IgG] of Acute infantile diarrhea with Norovirus, Adenovirus , Rotavirus infection and Control group.

Groups	No.	Mean	S. D	S. E	Min.	Max.
Acute infantile diarrhea with Norovirus infection	16	2325.9500	26.9408	19.0500	2306.90	2345.00
Acute infantile diarrhea with Enteric adenovirus infection	11	1310.5000	14.1421	10.0000	1300.50	1320.50
Acute infantile diarrhea with Rotavirus infection	20	2318.0500	60.8819	43.0500	2275.00	2361.10
Acute infantile diarrhea without Norovirus, Adenovirus or Rotavirus infection	33	1284.3500	23.4052	16.5500	1267.80	1300.90
Control	20	246.8700	33.1350	23.4300	223.44	270.30
Total	100	1497.1440	817.4822	258.5106	223.44	2361.10

## References :

- Adams C.P., Cohen E.J. and Albrecht J.(1984) Interferon treatment of adenoviral conjunctivitis. *Am J Ophthalmol.*;98:429-432
- Bartlett A.V., Moore M. and Gary G.W.(2005) Diarrheal illness among infants and toddlers in day care centers. I. *Epidemiology and pathogens J. Pediatr.*;107:495-502
- Bishop, R. F., G. P. Davidson, I. H. Holmes, and B. J. Ruck. (1973) Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterialgastroenteritis. *Lancet ii*: 1281-1283
- Brandt C.D., Kim H.W. and Vargosko A.J. (1999) Infections in 18,000 infants and children in a controlled study of respiratory tract disease: I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J. Epidemiol*;90:484
- Spencer MJ and Cherry JD(1981) Adenoviral infections, in Feigin RD, Cherry JD (eds): *Textbook of Pediatric Infectious Diseases*. Philadelphia, WB Saunders, pp 1279-1298.
- Brandt C.D., Kim H.W. and Rodriguez W.J.(1985) Adenoviruses and pediatric gastroenteritis. *J Infect Dis.*;151:437-443
- Drachman R.(2004) Acute infectious gastroenteritis. *Pediatr Clin North Am*;21:711-737.
- Gaudini G., Omani M. and Aied R. (1973) Isolation of enterovirus during an outbreak of acute haemorrhagic conjunctivitis in Algeria. *Arch Inst Pasteur Alger* 1977; 52: 119-28.
- Grohmann GS, Glass RI, Pereira HG, Monroe SS, Hightower A.W., and Weber R. (1993) Enteric viruses and diarrhea in adenovirus -infected patients. Enteric Opportunistic Infections Working Group. *N Engl J Med*;329:14–20.
- Hess, M. (2000) Detection and differentiation of adenoviruses: a review. In *Avian Pathol.*, Vol. 29, 195-206
- Hyman P.E and Ament M.E. (1982) Acute infectious gastroenteritis in children. *Pediatr Ann*;11:147-155
- Joncas J. and Pavilanis V. (1996) Diarrhoea and vomiting in infancy and childhood: viral studies. *Can Med Assoc J.*;82:1108-1113
- Kidd AH, Harley EH and Erasmus MJ(1985). Specific detection and typing of adenovirus types 40 and 41 in stool specimens by dot-blot hybridization. *J Clin Microbiol*;22:934-939
- Lew JF, Moe CL and Monroe(1991) Astrovirus and adenovirus associated with diarrhea in children in day care settings. *J Infect Dis*;164: 673-678
- Little JM, Centifanto YM, and Ufman HE. Immunoglobulins in human tears(1996) *Am J Ophthalmol*; 68: 898-905.
- Madeley C.R. (1986) The emerging role of adenoviruses as inducers of gastroenteritis. *Pediatr Infect Dis*;5:S63-S74
- Madeley C.R., Cosgrove B.P., Bell E.J. and Fallon R.J. (1977) Stool viruses in babies in Glasgow, I. Hospital admissions with diarrhoea. *J Hyg Camb*;78:261-273
- Middleton PJ, Szymanski MT and Petric M(1977). Viruses associated with acute gastroenteritis in young children. *Am J Dis Child*;131:733-737
- Romano A., Revel M. and Guarani-Rotman D. (2000) Use of human fibroblast-derived (beta) interferon in the treatment of epidemic adenovirus keratoconjunctivitis. *J Interferon Res*;1:95-100.
- Ruuskanen O, Meurman O and Sarkkinen H(1985). Adenoviral diseases in children: a study of 105 hospital cases. *Pediatrics*;76:79-83.

- Uhnnoo I., Wadell G., Svensson L. and Johansson M.E. (1984) Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J Clin Microbiol*;20:365-372.
- Unicomb LE, Banu N.N., Azim T., Islam A., Bardhan PK and FaruqueAS, (1998). Astrovirus infection in association with acute, persistent and nosocomial diarrhea in Bangladesh. *Pediatr Infect Dis J*; 17:611-4.
- Van R, Wun C., Ryan M.L., Matson D.O., Jackson L., and cking L.K. (1992). Outbreaks of human enteric adenovirus types 40 and 41 in Houston day care centers. *J Pediatr*;120:516-521.
- Welliver R.C., Sun M. and Losi D. (2008) Natural history of adenovirus infections in childhood Characteristics of immune response, abstracted. Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, p 254.
- Yolken R.H., Lawrence F., Leister F., Takiff H.E. and Strauss S.E.(2000) Gastroenteritis associated with enteric type adenovirus in hospitalized infants. *J Pediatr*;101:21-26.