Detection of Rotavirus Infection Among Children with Acute Diarrhea in Assiut Pediatric University Hospital: Genotying and Comparison Between Strips, EIA and RT-PCR

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Background: Group A rotavirus has been recognized as a leading cause of severe diarrheal disease in infants and young children worldwide and accounts for 20-25% of children diarrheal deaths per year. Defining the viral agents related to diarrhea will assist in providing an accurate estimate of disease burden within a community and will also be useful to assess the impact of vaccination whenever they become available. Epidemiological and molecular studies in many countries show complex patterns of change from year to year in the genotype of rotaviruses that cause diarrhea in children from the same geographical area. These data can be useful to select areas for vaccine trials and to serve as a baseline for identification of new strains, should they emerge.

Objectives: To investigate the role of group A rotavirus in acute diarrhea among infants and children under three years of age attending or admitted to Assiut Pediatric University Hospital and to compare between the strip test and Enzyme Immune Assay (EIA) in diagnosis of rotavirus infection using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) as a gold standard, beside defining the genotype of the detected strains using multiplex-PCR.

Methods: 88 children under the age of three years, presenting with acute diarrhea to Assiut Pediatric University Hospital between December 2005 and April 2006, were examined for group A rotavirus antigen in stool by a quick strip test and EIA. RT-PCR was also performed as a reference test. Twelve children of matched age and sex, without diarrhea, were also included (control group). All rotavirus positive samples by RT-PCR were also subjected to genotyping by multiplex PCR using a cocktail of primers specific for the most common genotypes of rotavirus in human (G1, G2, G3, G4, G8 and G9).

Results: Out of the 88 patients, 33 were rotavirus positive by strips (37.5%), 42 were rotavirus positive by EIA (47.7%) and 44 were positive by RT-PCR (50%). All control samples were negative by the three methods. 28 samples were positive by both strips and EIA and 44 samples were negative by both methods, whereas discordant results were obtained in 19 samples. Using the RT-PCR as a reference test, it was found that the strips failed to detect 13 out of 44 positive samples, while the EIA failed to detect nine out of these 44 positive samples. The sensitivity and specificity of strips versus RT-PCR were 70.5% and 95.5%, respectively, whereas those of EIA were 86.4% and 91%, respectively. Genotyping by multiplex PCR revealed that all the detected strains belong to G3 genotype.

Conclusion: The rate of infection with group A rotavirus among children with acute diarrhea differs according to the method used for detection (37.5% by strips, 47.7% by EIA and 50% by RT-PCR). The strips are more rapid, simple and specific than EIA, yet the EIA remains more sensitive in detecting rotavirus antigen in stool. All rotavirus strains detected belong to G3 genotype.

INTRODUCTION

Rotavirus is a leading cause of severe diarrheal disease in infants and young children worldwide and accounts for 20-25% of diarrehal deaths in children annually[(1)]. Globally, each year, rotavirus causes approximately 111 million episodes of gastroenteritis requiring only home care, 25 million clinical visits, two million hospitalizations and 600,000 deaths in children under five years of age. Despite similar indices of rotavirus infection in developed and developing countries, approximately 50% of rotavirus-related deaths occur in the poorest countries[(2)].

Rotaviruses are classified as a genus in the family Reoviridae. This genus currently has five species (rotavirus A to rotavirus E), with two possible additional species (rotavirus F and rotavirus G). The intact virion is 100 nm in diameter and is characterized by a distinct triple-layered capsid. Within the inner capsid is a third layer that encompasses the icosahedral core containing the virus genome. The genome consists of 11 segments of double-stranded RNA, which encodes six structural and six

non-structural proteins. The core proteins VP1, VP2 and VP3 and the inner capsid protein VP6 are encoded by gene segments 1, 2, 3 and 6, respectively. The outer capsid protein VP4 is encoded by segment 4 and the outer capsid protein VP7 is encoded by segment 7, 8 or 9, depending on the strain[3].

Rotaviruses have three important antigenic specificities, based on group, subgroup, and serotype. Group specificity is determined by the inner capsid protein VP6, and most epidemiologically significant rotaviruses of human and animal origin belong to group A (RV-A). This group is further classified into subgroups based on the specificities of epitopes that are also present on VP6. The majority of strains belong to either subgroup I or subgroup II. Serotype specificity is determined by the outer capsid proteins VP4 and VP7. The VP7 serotype is designated as a G serotype (G stands for glycoprotein) and the VP4 serotype is designated as a P serotype (P stands for protease-sensitive protein). The VP7 protein expresses the major neutralization antigen and is distinguishable by mean of both serological and genomic techniques into 14 G types, with good correlation between serological and genomic classification. VP4 expresses a major neutralization antigen, and the serological classification of the P type is much more difficult than genomic classification. To date, 13 P serotypes and 20 P genotypes have been defined but a precise correlation between the serological and genomic classification has not been made[4].

In temperate climates, viruses of types G1P[8], G3P[8], G4P[8] and G2P[4] are considered the most frequent genotypes, constituting more than 90% of the co-circulating strains. Animal-like genotypes such as the bovine-like G8 and the porcine-like G9 genotypes are observed at an increasing number among rotavirus infected children[5,6]. Genotypic studies of rotavirus strains among children less than two years of age in 1996-1998[7,8] suggested a substantial year-to-year variation in rotavirus genotypes, a high frequency of less common genotypes, a high frequency of mixed infections and finally a high frequency of the animal-like genotypes G8 and G9, especially among the mixed infections.

Rotavirus diarrhea can be effectively prevented by vaccination. The complexity of rotavirus strains, the genetic diversity and the different geographic distribution of rotavirus strains demand continuous worldwide surveillance of rotavirus strains before introduction and implementation of a vaccine. World Health Organization has been recommending local survey to assess rotavirus burden and the characterization of circulating strains as a part of strategies on implementing vaccination programs against this pathogen[9].

The aim of the present study was the detection of rotavirus (group A) infection among children with acute diarrhea attending or admitted to Assiut Pediatric University Hospital by using the quick strip test, enzyme immune assay (EIA) and reverse transcriptase-polymerase chain reaction (RT-PCR), and comparison between these three diagnostic methods. Genotyping of the detected rotavirus strains was done using the multiplex RT-PCR.

PATIENTS, MATERIALS AND METHODS

Study Population:

This study was conducted on 88 children, under the age of three years, presenting with diarrhea between December 2005 and April 2006. Of these patients, 37 were attending to the outpatient clinic of Assiut Pediatric University Hospital and 51 were hospitalized at the Gastroenterology Unit of the hospital. We also examined 12 healthy children with matched age and sex as a control group.

Specimen Collection:

Stool specimens were collected in clean containers (or diapers from infants and small children). Pre-designed questionnaire sheets contained information such as the name, age, gender, residence, duration of diarrhea, number of episodes of diarrhea and vomiting, presence of mucus or blood, dehydration status, type of feeding and personal hygiene.

Approximately one gm or one ml of stool specimen was suspended in 10 ml phosphate-buffered saline (PBS), pH 7.2, then centrifuged at 2000 rpm for 10 minutes. The supernatant was transported into three new Eppendorf tubes for examination by strips,

EIA, and RT-PCR and the tubes were stored at -20°C until examination.

Detection of Rotavirus Group A by Rapid Immunochromato-graphic Strip Test:

The RIDA Quick Rotavirus kit (R-Biopharm. AG, Germany, Cat. No. 0902) is a rapid immunochromatographic test for the qualitative screening of human faecal samples for detecting the presence of rotavirus group A antigen. 100 ul of stool sample were added to 1 ml extraction buffer and the tubes were mixed vigorously by vortex to homogenize the specimens then allowed to precipitate for at least three minutes until a clear supernatant was formed. The supernatant was transferred into another clean tubes and the test strips were immersed vertically into the sample tubes. After five minutes, the results were obtained. A maximum of two bands should appear, one red test band and one blue control band (antibodies directed against rotavirus are attached to red latex particles).

Detection of Rotavirus Group A by EIA:

An EIA kit (R-Biopharm. AG, Germany, RIDA Screen Rotavirus kit, Cat. No. 0901) was used to detect rotavirus antigen group A in stool samples. This kit employs monoclonal antibodies against the capsid protein of gene 6 (VP6) in a sandwich-type method[10]. 100 ul of the processed stool samples, negative and positive controls were added to the microplate wells and 100 ul of peroxidase conjugate were added simultaneously to each well. The plate was incubated for 60 minutes at room temperature then washed five times with 300 ul of diluted wash buffer. 100 ul of substrate (urea peroxide/TMB) were added to each well and incubated for 15 minutes at room temperature. 50 ul of stop solution (1N H_2SO_4) were added to each well then the absorbance of each well was read at 450 nm filter in ELISA reader. The cut-off was calculated (cut-off = negative control absorbance + 0.15) and the samples were considered positive if their extinction was more than 10% above the calculated cut-off.

Determination of Rotavirus RNA by RT-PCR (Round-I amplification):

A) Extraction of Rotavirus ds-RNA: Rotavirus ds-RNA was extracted from 140 ul of clarified stool samples using the QIAamp viral RNA minikits (QIACEN, Basel, Swizerland, Cat. No. 52904) according to the technique described by the manufacturer. The extracted RNA was stored at -20°C until the amplification was performed.

B) RT-PCR (QIAGEN one-step RT-PCR kit, Cat. No. 210210): Reverse transcription and PCR (amplification) were performed in a single step using enzyme mix for both reverse transcription and PCR amplification (omniscript reverse transcriptase, sensiscript reverse transcriptase and hot start Taq DNA polymerase). The primers used were the Beg9 and End 9 primers (Operon) which are specific for group A rotavirus and amplify the full length of VP7 gene (G types)[11].

(Beg 9 5'-GGCTTTAAAAGAGAGAATTTCCGTCTGG-3'
End 9 5' GGTCACATCATACAATTCTAATCTAAG-3')

40 ul master mix was prepared for each sample as the following: 22 ul RNase free water, 2 ul dNTp mix, 10 ul 5x buffer, 2 ul Beg 9 primer (0.4 µM), 2 ul End 9 primer (0.4 µM) and 2 ul one step RT-PCR enzyme mix. 10 ul of template RNA were added to the prepared master mix in PCR tubes and covered with one drop of mineral oil. The PCR tubes were placed in the thermal cycler using the following program: RT at 50°C for 30 min., initial denaturation at 95°C for 15 min., 40 cycles of denaturation (94°C), primer annealing (45°C) and extension (72°C) for one min. each, then a final step of extension at 72°C for 10 min. The amplified products were stored at -20°C until used.

C) Detection of Amplified DNA by Agarose gel Electrophoresis: Seven microliters of the amplification products were mixed with three microliters loading buffer and electrophoresed on 1.5% agarose gel in Tris-borate-EDTA buffer for one hour at 100 v. (The gel was stained with ethidium bromide 0.5 ug/ml). A negative control (water) and 100-bp DNA ladder (KomaBiotech, Korea) were also loaded with each run. The gel was visualized under ultraviolet light and the positive samples produced one 1062 bp DNA fragment.

Genotyping of Rotavirus by Multiplex-PCR (Round-II amplification):

Rotavirus positive samples by Round-I amplification were subjected to genotyping by multiplex-PCR using Qiagen Taq. PCR master mix (Cat. No. 201443). The master mix contains Taq DNA polymerase (2 units), 2x PCR buffer, 4 mM $MgCl_2$ and 400 µM of each dNTp. The primers used for G genotyping were the Beg 9-primer (antisense) against the G1, G2, G3, G4, G8 and G9 primers[12]. For each sample, primers mix containing 0.4 µM of each primer was prepared as follows: 2.6 ul antisense primer, 2.6 ul G1 primer, 2.0 ml G2 primer, 2.8 ul G3 primer, 3.2 ul G4 primer, 2.6 G8 primer, 2.4 ul G9 primer and 3.8 ul distilled water. The final volume of diluted primers for each sample was 22 ul to which 25 ul of PCR master mix were added giving a final volume of 47 ul/sample. Three microliters of template DNA were added to the prepared mix in PCR tubes and covered with one drop of mineral oil, then amplified in the thermal cycler using the following program: initial denaturation at 94°C for 15 min, 40 cycles of denaturation (94°C), annealing (55°C) and extension (72°C) for one minute each. Then, additional extension at 72°C for 10 min after the 40 cycles. The amplified products were stored at -20°C until examined by agarose gel electrophoresis. The genotype was determined by comparing the site of the amplified DNA fragment to the different bands of the DNA ladder. The DNA fragments of the different genotypes are as follows: 749 bp for G1, 652 bp for G2, 374 bp for G3, 583 bp for G4, 885 bp for G8 and 306 bp for G9.

Statistical Methods:

Sensitivity, specificity, positive and negative predictive values were calculated for strip test and EIA against RT-PCR using the following equations[13].

$$\text{Sensitivity} = \frac{\text{True +ve}}{\text{True +ve + False -ve}} \times 100$$

$$\text{PPV} = \frac{\text{True +ve}}{\text{True +ve + False +ve}} \times 100$$

$$\text{Specificity} = \frac{\text{True -ve}}{\text{True -ve + False +ve}} \times 100$$

$$\text{NPV} = \frac{\text{True –ve}}{\text{True -ve + False -ve}} \times 100$$

Chi-square (χ^2) test and T-test were used to analyze data using SPSS (Statistical Package of Social Science, Version 10. Inc., Chicago, USA). P-value <0.05 was considered significant.

RESULTS

This study was conducted on 88 patients presenting with acute diarrhea and 12 control subjects without diarrhea of matched age and sex. The age of the patients ranged from two to 36 months (mean ± SD = 9.17 ± 5.9 months). 58 patients were males (65.9%), their mean age ± SD was 8.4 ± 5.1 months. 30 patients were females (34.1%), their mean age ± SD was 10.6 ± 6.7 months (P> 0.05). Out of 88 patients, 51 patients were hospitalized (58%), their mean age ± SD was 10.12 ± 5.14 months, 35 of them were males (68.6%) and 16 were females (31.4%). 37 patients were non-hospitalized attending the outpatient clinic (42%), their mean age ± SD was 7.86 ± 6.4 months, 23 of them were males (62.2%) and 14 were females (37.8%). The age and sex distribution of patients is shown in Table(1).

Table (1): Age and sex distribution of patients.

Age group (months)	Male n (%)	Female n (%)	Total n (%)
2 < 6	21/26 (80.8)	5/26 (19.2)	26 (29.5)
6-12	22/35 (62.9)	13/35 (37.1)	35 (39.8)
12-36	15/27 (55.6)	12/27 (44.4)	27 (30.7)
Total	58 (65.9)	30 (34.1)	88 (100)

P = 0.13 (NS).

The duration of diarrhea was less than five days in 65.9% of patients, from five to ten days in 27.3% of patients and more than 10 days in only 6.8% of patients. The clinical criteria of patients is shown in Table (2).

Table (2): Clinical criteria of patients.

Clinical criteria	Inpatients (n= 51)		Outpatients (n= 37)		Total (n= 88)	
	No.	%	No.	%	No.	%
Fever	32	62.7	14	37.8	46	52.3
Vomiting	45	88.2	28	75.6	73	83.0
Mucous in stool	8	15.6	7	18.9	15	17.0
Blood in stool	0	0	1	2.7	1	1.1
Upper respiratory infection	22	43.1	18	48.6	41	46.6
Bad personal hygiene	32	62.7	24	64.8	56	63.6
Dehydration	32	62.7	8	21.6	40	45.5
No. of defecation						
<5 times	4	7.8	7	18.9	11	12.5
5-10 times	21	41.2	22	59.4	43	48.9
>10 times	26	51.0	8	21.7	34	36.6

Results of Detection of Rotavirus (Group A) in Stool by Strips, EIA and RT-PCR:

Out of the 88 patients, 33 were positive by strips (37.5%), 42 were positive by EIA (47.7%) and 44 samples were positive by RT-PCR (50%). All control samples gave negative results by the three methods. On comparing between the results of strips and EIA, it was fount that out of the 88 samples, 28 samples were positive and 41 samples were negative by both methods. Discordant results were observed in 19 samples; five samples were negative by EIA and positive by strips, while 14 samples were negative by strips and positive by EIA (Table 3). Using the RT-PCR as a reference test, it was found that the strips failed to detect 13 out of 44 positive samples, while the EIA failed to detect only 6 out of 44 positive samples.

Table (3): Results of rotavirus detection in stool by quick strip test and EIA.

Method	Strip –ve	Strip +ve	Total
EIA –ve	41	5	46
EIA +ve	14	28	42
Total	55	33	88

Sensitivity and Specificity of Strips and EIA Versus RT-PCR:

Table (4) displays the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of strips and EIA versus RT-PCR.

Table (4): Sensitivity and specificity of rotavirus strip test and EIA when tested against RT-PCR.

Test	RT-PCR		
	Positive	Negative	Total
Rotavirus strip test*			
Positive	31	2	33
Negative	13	42	55
Total	44	44	88
Rotavirus EIA**			
Positive	38	4	42
Negative	6	40	46
Total	44	44	88

*Sensitivity = 31 / 44 x 100 = 70.5%
Specificity = 42 / 44 x 100 = 95.5%
PPV = 31 / 33 x 100 = 94%
NPV = 42 / 55 x 100 = 76.4%

**Sensitivity = 38 / 44 x 100 = 86.4%
Specificity = 40 / 44 x 100 = 91%
PPV = 38 / 42 x 100 = 90.5%
NPV = 40 / 46 x 100 = 87%

Relation Between Rotavirus Infection and Clinical Criteria of Patients (Chi-square test):

Among the 44 rotavirus positive patients (by PCR), infants between six and 12 months old had the highest frequency of rotavirus infection (20/44 = 45.4%), while infants less than six months old had the lowest frequency of infection (8/44 = 18.2%). The frequency of rotavirus infection in age group 12-36 months was 36.4% (16/44) (P = 0.05) (Table 5). Rotavirus infection rate was higher in males (34/44 = 77.2%) than in females (10/44 = 22.8%), this difference was statistically significant (P = 0.04) (Table 5).

Table (5): Age and sex wise analysis of rotavirus infection among children with acute diarrhea.

	Rotavirus +ve (n= 44)	Rotavirus –ve (n= 44)	Total (n= 88)	P value
Age group				
< 6 ms	8 (18.2%)	18 (40.9%)	26 (29.5%)	0.05
6-12 ms	20 (45.4%)	15 (34.1%)	35 (39.8%)	
12-36 ms	16 (36.4%)	11 (25%)	27 (30.7$)	
Gender				
Male	34 (77.2%)	24 (45.5%)	58 (65.9%)	0.04
Female	10 (22.8%)	20 (54.5%)	30 (34.1%)	

Among the hospitalized patients (n= 51), 30 patients were rotavirus positive (58.8%), whereas among the outpatients (n= 37), only 14 patients were rotavirus positive (37.8%) (P = 0.08). The relation between rotavirus infection and other clinical criteria including duration of disease, fever, vomiting, blood and mucus in stool, dehydration, number of evacuation are shown in Table (6). Among rotavirus positive patients, fever and dehydration were observed in 52.3%, vomiting was observed in 84.1% and evacuation more than ten times per day was observed in 38.6%.

Table (6): The statistical association of clinical criteria with rotavirus infection among 88 children suffering from acute diarrhea in Assiut Pediatric University Hospital, 2006.

Clinical criteria	Rotavirus infection			
	All patients No/88 (%)	RV +ve No/44 (%)	RV -ve No/44 (%)	P-value
Fever	46 (52.3%)	23 (52.3%)	23 (52.3%)	NS
Vomiting	73 (83%)	37 (84.1%)	36 (81.8%)	NS
Blood in stool	1 (1.1%)	1 (2.3%)	0	
Mucous in stool	15 (17%)	6 (13.6%)	9 (20.5%)	NS
Upper resp. inf.	41 (46.6%)	23 (52.3%)	18 (40.9%)	NS
Bad per. hygiene	58 (63.6%)	32 (72.8%)	24 (54.5%)	NS
Dehydration	40 (45.5%)	23 (52.3%)	17 (38.6%)	NS
No. of defecation				
< 5 times / day	11 (12.5%)	4 (9.1%)	7 (16%)	NS
5-10 times / day	43 (48.9%)	23 (52.3%)	20 (45.4%)	NS
> 10 times / day	34 (38.6%)	17 (38.6%)	17 (38.6%)	NS
Duration of disease				
< 5 days	58 (65.9%)	32 (72.7%)	26 (59.1%)	NS
5-10 days	24 (27.3%)	10 (22.7%)	14 (31.8%)	NS
> 10 days	6 (6.8%)	2 (4.6%)	4 (9.1%)	NS

RV = Rotavirus NS= not significant.

Genotyping of Rotavirus by Multiplex RT-PCR:

Out of the 88 patients with acute diarrhea, rotavirus was detected in 44 samples by TR-PCR (1st round of amplification) using the Beg 9 and End 9 primers which amplify the whole length of VP7 gene (Fig. 1).

All positive amplification products we re further amplified by a 2nd round of amplification using multiplex of G-specific primers that identify the following VP7 genotypes: G1, G2, G3, G4, G8 and G9. Genotype G3 was detected in all positive samples which gave one band at 374 bp DNA fragment on examination of ethidium bromide stained gel under ultraviolet light (Fig. 2).

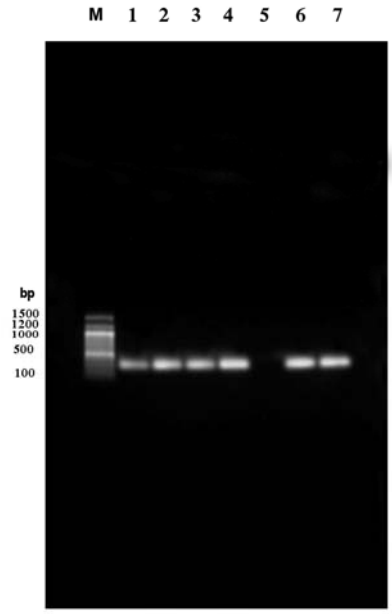

Fig. 1: First round PCR.
Amplification products of the VP7 gene; Lines 1 to 3 and 5 to 7 show first round amplification products (1,062 bp) which indicates group A rotavirus. Line 4 indicates negative result. DNA ladder was used as a molecular weight marker (M).

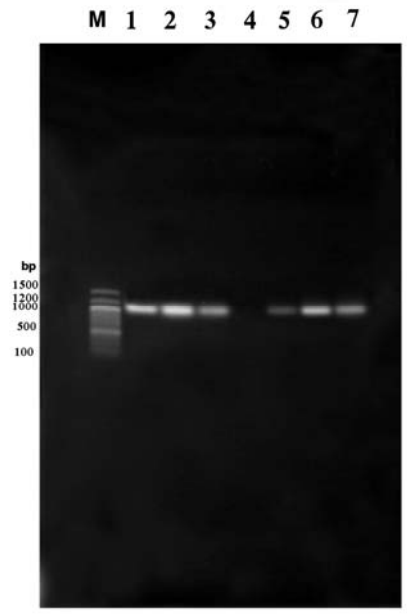

Fig. 2: Amplification products of the second round PCR for genotyping; lines 1 through 4 and 6 to 7 show genotype G3 (374bp). Line 5 indicates negative result.

DISCUSSION

Acute diarrhea is the most prevalent disease of childhood and is one of the most important causes of mortality in children under five years of age in developing countries[4]. Defining the viral agents related to diarrhea will assist in providing an accurate estimate of disease burden within a community. This will allow for a proper assessment of the contribution of each virus to morbidity. The availability of accurate information on the aetiology of viral diarrhea will also be useful when it comes to assessing the impact of vaccinations whenever they become available. In many countries human rotaviruses are recognized as the main cause of acute gastroenteritis in infants and children[1]. In the present study, 88 patients less than three years of age, suffering from acute diarrhea and attending or admitted to Assiut Pediatric University Hospital between December 2005 and April 2006, were examined for rotavirus infection in stool by rapid strip test, EIA and multiplex RT-PCR. 37 patients were non-hospitalized (42%) and 51 patients were hospitalized (58%). Out of the 88 patients, 58 were males (65.9%) while 30 were females (34.1%).

Rate of Infection:

Rotavirus was detected in 33/88 (37.5%) of patients by strips, 42/88 (47.7%) of patients by EIA and 44/88 (50%) of patients by RT-PCR. In a hospital-based study in Cairo[14], rotavirus was detected in 33% of infants with acute complicated diarrhea (associated with severe dehydration, bleeding, pneumonia or seizures). In rural Egypt, 44% of children experienced at least one bout of rotavirus diarrhea by the age of three years[15,16]. Another study in Cairo[17], identified rotavirus in 35.6% of neonates and infants less then one year of age by EIA over a 15-month period from August 1992 to October 1993. None of the previous studies in Egypt used the RT-PCR to evaluate the rate of infection by rotavirus. RT-PCR assays are used more and more frequently in large scale investigations to understand the epidemiological features of viral diarrhea among children. This is done not only because it is - at least by some investigators - considered to be more sensitive than EIA and latex, but also because it can be used to further genotype and compare to homogeneity of the circulatory virus strains in different years and regions[18,19].

High infection rate was also observed in China[20], where rotavirus group A was detected in 59% of stool samples from children less than 5 years of age by EIA and/or RT-PCR in winter season. In Rio de Janeiro, Brazil, group A rotavirus was detected in 48% of children admitted to the hospital between February and September 2004 by polyacrylamide gel electrophoresis (PAGE) and EIA[21]. In Mexico, rotavirus was detected in 48.7% of patients with acute diarrhea by PAGE (96% under 4 years of age)[22]. In India, rotavirus was detected in 37.1% of children less than 4 years by PAGE, and in Paraguay it was detected in 31.8% of children with acute diarrhea less than three years by EIA and/or PAGE[23,24]. In Taiwan, rotavirus was detected in 31% of stool samples from acute diarrhea children 13 years or younger by PCR[25].

Lower rates of group A rotavirus infection were observed in other studies; 22% in South India[26], 17% in Botswana[27], 17% in Tunisia[28], 15.3% in Tehran, Iran[29] and 10% in Mekkah, Saudi Arabia[30].

Age:

In the present study, the highest frequency of infection with group A rotavirus was observed in the age group 6 to 12 months (54.4%), while the lowest rate of infection was observed in the age group less than six months (18.2%). These results are consistent with other studies carried out in Egypt and other developing countries, where the highest frequency of infection was observed in the age group between six and 12 months[26-33].

In the contrary to all previous studies, Coluchi *et al.*[24] revealed that the highest frequency of rotavirus infection was between one to three years of age.

Gender:

In the present study, boys were more frequently affected than girls (P = 0.04), 77.2% of rotavirus patients were boys and 22.8% were girls. Some studies also reported higher rate of infection with rotavirus among males[22]. However, in other studies, no

association could be discerned between male and female children analyzed[26].

Hospitalization:

In the present study, the frequency of rotavirus infection was higher among hospitalized (severe infection) rather than non-hospitalized patients (58.8% compared to 37.8%). A statistically significant relation was found when rotavirus was detected by EIA (P= 0.02). Our results were in agreement with many other studies. Coluchi *et al.*[24] have found that rotavirus infection was detected in 42.5% of hospitalized patients compared with 12.6% of outpatients. Zarnani *et al.*[29] detected rotavirus infection in 21.9% of hospitalized patients compared to 12% in outpatients (P< 0.001). Fang *et al.*[34] identified rotavirus infection in 51% of inpatients compared to 29% of outpatients.

Clinical Criteria:

In this study, the clinical criteria of patients did not differ significantly between rotavirus positive and negative patients. This may be explained by the presence of other infectious causes of severe diarrhea in children associated with vomiting, fever and dehydration. However, Bern *et al.*[35] have found significant association between rotavirus infection and vomiting, watery diarrhea, fever and severe dehydration. Zarani *et al.*[29] have also found that rotavirus infection was associated with watery diarrhea, fever, vomiting and dehydration.

Genotyping of Rotavirus:

The four predominant rotavirus genotypes G1-4 comprise nearly 83% of all rotavirus infections in the world[24,26,35-39]. Genotype 9 has emerged in many studies in different parts of the world[23,39-41] and was detected at higher frequency in Taiwan[25], Bangladesh[32] and Brazil[21]. G1 was reported as the predominant genotype allover the world in many studies[21,27,28,37-40]. However, G2 was predominant in Guinea-Bissau[42] and South India[26], and genotype G4 was the predominant type in Paraguay[24].

In this study, genotype G3 was identified in all rotavirus positive samples, no other genotypes could be detected. This may be due to the limited period of time (five months only) and limited number of patients. Other genotypes could be detected during other seasons of the year and could differ from one year to another. Earlier studies in Cairo, Egypt (1992-1993)[17] revealed that G1 and G4 (serotypes) were the most frequent types of rotavirus in children with diarrhea (17.7%, each). G3 was detected in 6.5% of rotavirus positive samples and it was the third common serotype in infants between six and twelve months. There was a seasonal variations in the prevalent serotype; G1 and G4 were found most frequently from August to October in neonates and from September to December or June to August in older infants. Serotype G3 had a uniform distribution in infants throughout the years. Naficy *et al.*[31] detected rotavirus infection in AbuHomos, Egypt. They have found that 89% of rotavirus positive isolates were serotypes G1 and G2. In rural Bangladesh, Bern *et al.*[35] detected rotavirus serotypes G1,2,3 and G4 with more severe dehydration associated with G2 and G3 serotypes, but the difference did not appear to be of major clinical importance. Kasule *et al.*[27] in Botswana (1998-2001) detected rotavirus genotype G1 by RT-PCR in 59% of rotavirus positive samples. Mixed G1 and G3 could be detected in 5% of positive cases. Genotype G3 was also detected in South India (1995-1999)[26], in Spain (1998-2002)[39] and in Netherlands (1997-2001)[40].

The factors that determine the identity of rotavirus strains in circulation among human population, as well as their well-known temporal and periodic variations, deserve more intense investigations that will allow them to be clarified and better understood. Ongoing surveillance of seasonal rotavirus genotype patterns is needed to monitor the spread of new or emerging genotypes like G9 and G5. Such information could influence the strategy for development of new generations of rotavirus vaccines.

Comparison Between Rotavirus Detection in Stool by Strips, EIA and RT-PCR:

There is a great controversy concerning the most feasible test for screening of rotavirus infection. There is a need for rapid and sensitive rotavirus detection methods in routine diagnostic laboratories, many of which perform rotavirus group antigen detection using either latex agglutination assay or EIA. We set out to compare the sensitivity and specificity of a current commercial quick strip test and an EIA for

detection of group A rotavirus in stool against the RT-PCR as a reference test. Among the 44 positive samples by RT-PCR, the strips failed to detect 13 positive samples (29.5%) whereas the EIA failed to detect only six positive samples (13.6%). Out of the 44 negative samples by RT-PCR two false positive results were obtained by the strips (4.5%), while four false positive results were obtained by EIA (9%). The quick strip test showed higher specificity than EIA (95.5% compared to 91%), but lower sensitivity than EIA (70.5% compared to 86.4%).

Contradictory results were obtained by Wilhemi *et al.*[42] 228 faecal samples where evaluated for group A rotavirus by strips, latex agglutination and EIA. Discordant results were resoluted by RT-PCR. EIA was found to be less sensitive than strips (96% compared to 99%), but more specific (99% compared 96%). Another study conducted by Dewar *et al.*[43] compared the sensitivity and specificity of latex agglutination and rotavirus strip test with EIA as a gold standard. The strips failed to detect nine out of 90 positive samples by EIA, but all negative samples by EIA where also negative by strips (sensitivity 88% and specificity 100%).

Steyer *et al.*[40] examined 99 stool samples for rotavirus by EIA and RT-PCR. The sensitivity and specificity of EIA against RT-PCR were 89.8% and 97.9%. Altindis *et al.*[44] examined the sensitivity and specificity of EIA against PAGE. The sensitivity of EIA was 100% and its specificity was 99.2%. An earlier study conducted by Buesea *et al.*[45] compared RT-PCR, EIA, PAGE and electron microscopy, they found positivity rates of 30% for PCR, 29% for EIA, 26.8% for PAGE and 25.4% for electron microscopy (RT-PCR was the most sensitive test).

Our results suggest that although the strip test is rapid, simple and less costly, yet the EIA remains more sensitive as a screening test for rotavirus infection. It is appropriate that laboratory personal regularly monitor available kits to find the most sensitive assay. Accurate and rapid diagnosis of rotaviral infection should reduce unnecessary use of antibiotics in patients infected only with rotavirus. This would improve treatment, overall costs would be lowered and inappropriate use of antibiotics and resulting resistance would be reduced. Rapid rotavirus diagnostic capacity with a quick turn around time would facilitate containment of infected patients, improving infection control and preventing nosocomial outbreaks.

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الكشف عن عدوي فيروس "روتا" بين الأطفال المصابين بالإسهال الحاد في مستشفي أسيوط الجامعي للأطفال: التصنيف الجيني ومقارنة بين اختبار الشرائط ، القياس المناعي الانزيمي ، النسخ العكسي / تفاعل البلمرة المتسلسل

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تعتبر المجموعة A من فيروس روتا سبباً رئيسياً للإسهال الحاد بين الأطفال في العالم كله ، فهي مسئولة عن ٢٠ إلي ٢٥% من الوفيات بسبب الإسهال في كل عام. ان تحديد نوع الفيروس المسبب لحالات الإسهال سوف يساعد علي التقدير الدقيق لما يسببه هذا الفيروس من ضرر في المجتمع وكذلك يساعد علي تحديد تأثير اللقاح المستخدم حينما يكون متاحاً. تشير الدراسات الجزيئية إلي أن التركيب الجيني للفيروس يتغير من عام إلي آخر في نفس المنطقة الجغرافية وهذا من الأهمية لاختيار اللقاح المناسب الذي يحتوي علي الأنواع الجينية المختلفة المرتبطة بالمرض وتحديد الأنواع الجينية الجديدة التي قد تظهر.

الأهداف: تقصي دور فيروس روتا في حالات الإسهال الحاد بين الأطفال الأقل من ثلاث سنوات المقيمين او المتقدمين للعيادات الخارجية بمستشفي أسيوط الجامعي للأطفال والمقارنة بين طريقة الشرائط والقياس المناعي الانزيمي (EIA) في التشخيص وذلك بالمقارنة بطريقة النسخ العكسي / تفاعل البلمرة المتسلسل (RT-PCR) ، ذلك بالإضافة إلي تحديد النوع الجيني للفيروس.

الطريقة: تمت الدراسة علي ٨٨ طفلاً أقل من ثلاث سنوات يعانون من الإسهال الحاد (سواءاً نزلاء مقيمين أو مترددين علي العيادات الخارجية) بمستشفي أسيوط الجامعي للأطفال في الفترة من ديسمبر ٢٠٠٥ إلي أبريل ٢٠٠٦. تم الكشف عن الأنتجين الخاص بمجموعة (A) لفيروس روتا في عينات البراز الخاص بالمرضي وكذلك ١٢ من الأطفال الأصحاء (مجموعة حاكمة) بطريقة الشرائط وEIA بالمقارنة بطريقة الـ RT-PCR بإعتباره الأكثر دقة.

النتائج والاستنتاجات: أظهرت النتائج وجود الفيروس في عينات البراز الخاصة بالمرضي بنسبة ٣٧,٥% بطريقة الشرائط ، ٤٧,٧% بطريقة EIA ، ٥٠% بطريقة الـ RT-PCR. وكان النوع الجيني G3 هو النوع الوحيد الموجود بهذه العينات. وأظهرت الدراسة أن طريقة الشرائط وإن كانت أكثر سرعة وبساطة إلا أنها أقل في الحساسية عن طريقة الـ EIA في الكشف عن فيروس روتا.