Genotyping and Severity of Rotavirus Infection among Infants and Children with Acute Diarrhea

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ABSTRACT

Introduction: Rotavirus (RV) belongs to the Reoviridae virus family and the virion comprises of three concentric protein layers. Worldwide, rotavirus (RV) is the most common cause of severe gastroenteritis (G.E.) among infants and young children especially in those countries which has not launched a RV vaccination program and approximately 40% of hospitalized patients suffering from gastroenteritis were infected with RV. **Objective:** To determine the prevalence of rotavirus infections, genotypes and degree of severity of its acute diarrhea in infants and children attending the children hospital, at Zagazig University Hospitals. **Patients and methods:** this study was done on 140 patients admitted to Children Hospital, Zagazig University suffering of gastroenteritis, Vesikari clinical severity score was done and stool sample was taken from patients, which was tested for RV by dipstick method and positive patients underwent genotyping by immunochromatography using PCR technique.

Results: Among the studied group 128 patients (91.4%) showed positive results for rotavirus detection by dipstick analysis of stool, while only 12 patients (8.6%) were negative. The Vesikari score of severity ranged from 9 to 15 with median of 12 and its mean ± SD was 11.9±1.3. G3 and P8 were the most types in the examined patients.

Conclusion: rotavirus is still the main cause of severe gastroenteritis that requires hospital admission. G3 and P8 are the most detectable genotypes.

Keywords: Diarrhea, Genotyping, Infants and Children, Rotavirus.

INTRODUCTION

Worldwide, rotavirus (RV) is the most common cause of severe gastroenteritis among infants and young children especially in those countries which has not launched a RV vaccination program [(1)]. Every child encounters at least one episode of RV gastroenteritis by the age of 5 years. Each year, about 2 million subjects have to be hospitalized for developing severe RV gastroenteritis while about 25 million patients seek medical help by visiting a physician's office or clinic and 111 million cases require care at home [(2)]. While diarrhea is the second most common cause of fatal childhood illness, about 1.34 million deaths occur worldwide among children aged less than 5 years due to RV [(3)].

Though the incidence of RV infection among children in developed and developing countries is similar, outcomes vary widely with 82% of fatalities estimated to occur in developing countries. Most death occurs in low- and middle-income countries, such as Egypt [(4)]. RV can be detected in high concentrations in the stool of children suffering from gastroenteritis. Control measures such as improved sanitation is not effective in preventing this disease [(5)].

Several studies performed in the Middle East showed that approximately 40% of hospitalized patients suffering from gastroenteritis were infected with RV [(2, 6)]. Rotavirus (RV) belongs to the Reoviridae virus family and the virion comprises of three concentric protein layers [(7)]. The outer capsid consists of two proteins, VP7 and VP4 that are used to classify rotavirus strains into G (glycoprotein) and P (protease sensitive) genotypes, respectively [(8)]. Of the 12 G and 15 genotypes known to infect humans, genotypes G1P81, G2Pl4, G3P8, G4P8 and G9P8 cause over 90%of rotavirus disease worldwide [(9)].

The aim of this work was to determine the prevalence of rotavirus infections, genotypes and degree of severity of its acute diarrhea in infants and children attending the Children Hospital, at Zagazig University Hospitals.

SUBJECTS, MATERIALS AND METHODS

This study was a cross-sectional study conducted in Pediatric Department, Zagazig University Hospitals, Medical Microbiology and Immunology Department and in Medical Scientific and Research Centre, Faculty of Medicine, Zagazig University Hospitals.

For one year surveillance, the data and the stool samples were gathered from January 2019 to January 2020 (in November, December, January months which was the peak of RV). A total of 140 stool specimens were collected from admitted patients diagnosed with acute diarrhea in Pediatric Department, Zagazig University Hospitals. The stool samples were from the consecutive patients in this study period. Case enrollment was done on the basis of inclusion and exclusion criteria.

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Ethical approval:

An approval of the study was obtained from Zagazig University academic and ethical committee. Informed consent was obtained from written informed consent was taken from parents for participation in the study. After being informed about the aims and process of the study as well as applicable objectives.

Inclusion criteria: Infants and children aged from one month to five years presented with gastroenteritis or acute diarrhea who were not vaccinated with rotavirus vaccine (non-compulsory vaccine in Egypt).

Exclusion criteria: Children with chronic and/or persistent diarrhea, which was defined as diarrhea that lasted for more than two weeks.

All study subjects underwent: Thorough history taking including personal history (age, sex, name, number of children, education and occupation of the parents) and present history (symptoms, duration of symptoms, temperature of the child, hydration state). Physical examination and laboratory investigations including: Ag detection of rotavirus by immunochromatography and genotyping (for Ag positive samples) by PCR technique.

Physical examination: for detection of severity of diarrheal disorder using Vesikari score of severity as in table 1 where Mild cases < 7, Moderate cases 7- 10, Severe cases 11- 20 **[10].**

Table (1): Vesikari score of G.E. severity

Parameter	1	2	3
Diarrhea			
Maximum number of stool per day	1-3	4-5	≥6
Diarrheal duration (days)	1-4	5	≥6
Vomiting			
Maximum number of vomiting episodes per day	1	2-4	≥5
Vomiting duration (days)	1	2	≥3
Temperature	<38.5	38.5-38.9	≥39
Dehydration	N/A	1-5%	≥6%
Treatment	N/A	Rehydration	Hospitalization

- Stool samples collection: Stool samples were collected as watery diarrhea in sterile containers. On reaching the lab each sample was divided into two Falcon tubes (15 ml) (one frozen at -20ºC for immunochromatography test and the other at -80ºC for PCR genotyping).
- Rotavirus antigen detection by **RIDA® QUICK Rotavirus (dipsticks) kit:** For in vitro diagnostic use. This test is a quick immunochromatographic test for the qualitative determination of rotaviruses in stool samples (Art. No.: N0902, Germany). Procedures and interpretation of results were done according to the manual instructions.
- Samples positive for rotavirus Ag by immunochromatographic test underwent RNA extraction and PCR genotyping.
- **Viral RNA extraction by QIAamp® viral RNA minikit (Qiagen)**; (Extraction kit for viral RNA) (Cat. No. 52904, The Netherlands). Procedures were done according to the manual instructions.
- **Rotavirus genotyping:** Reagents and materials needed:
  - Qiagen® onestep RT-PCR kit (Cat. No.: 210210, The Netherlands).
  - Primers (9con1,9con2,9T1,9T2,9T3P,9T4,9T9B,con3,con2 ,1T1,2T1,3T1,4T1,5T1,ND2)
  - Taq DNA polymerase 250µ, 10x PCR buffer (HVD Egypt).
  - Deionized water.
  - For genotyping of rotavirus 2 types of genotyping were done (genotyping for P proteins (P genotyping) and genotyping for G proteins (G genotyping). (According to the WHO protocol). The protocol for genotyping was provided by the WHO Rotavirus Collaborating Center, Atlanta, Georgia, USA and also used by the Eastern Mediterranean Regional Rotavirus Laboratory **[11].**

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). P value < 0.05 was considered significant.

RESULTS

Our sample consisted of (140) patients, 87 male (62.1%) and 53 female (37.9%) with age ranges from 1 to 50 months (mo). fifty infants from 1 to 12 months represent (35.7%), 38 infant from 12 to 24 months (27.1%), 36 children from 24 to 36 mo (25.7%) and 16 children from 36 to 50 mo (11.4%). The Vesikari score of severity ranged from 9 to 15 with median of 12 and

its mean ± SD was 11.9±1.3, with mean duration of diarrhea of 2.8±0.8 days and mean duration of vomiting 2.1±0.6 days. Diarrheal duration ranged from 1 to 5 days, vomiting continued from 1 to 3 days and hospital stay ranged from 2 to 6 days. 87.1% of our patients showed severe dehydration while 22.9 had mild dehydration. Among the studied group 128 patients (91.4%) showed positive results for rotavirus detection by dipstick analysis of stool, while only 12 patients (8.6%) were negative.

There was no statistically significant difference between rota +ve cases and rota –ve cases regarding age, sex, number of motions, duration of diarrhea or vomiting but there was statistically significant difference between them regarding weight, degree of dehydration, Vesikari score and duration of stay in hospital (Tables 2, 3 and 4).

Table (2): Comparing age and sex between positive and negative Rota virus in the studied group

Variable	Positive (128)		Negative (12)		P-value
	Mean ± SD Median (Range)		Mean ± SD Median (Range)		
Age (months)	20.7±6.5 18.5 (8-35)		20.1±5.4 19 (6-50)		0.8
	F (128)	**%**	**F (12)**	**%**	**P-value**
Sex:					
Male	81	63.3	6	50	0.4
Female	47	36.7	6	50	

F=frequency

Table (3): Comparing clinical data between positive and negative rotavirus in the studied group

Variable	Positive (128)	Negative (12)	P-value
	Mean ± SD Median	Mean ± SD Median	
Vesikari score of Severity	13.9±1.4 13	10.7±0.8 10.5	**<0.001**
Motion numbers	5.7±1.2 6	5.2±0.4 5	0.2
Diarrheal duration (days)	2.8±0.87 3	2.7±0.45 3	0.6
Vomiting duration(days)	2.1±0.6 2	2.2±0.4 2	0.3
Hospital stay(days)	3.5±1.3 3	2.5±0.5 2.5	**0.01**

Table (4): Comparing weight and dehydration degree between positive and negative Rota virus in the studied group

Variable	Positive		Negative		P-value
	F (128)	%	F(12)	%	
Weight					
Normal	27	21.1	6	50	**0.02**
Underweight	101	78.9	6	50	
Dehydration					
Moderate	18	14.1	6	50.0	**<0.002**
Severe	110	85.9	6	50.0	

F=frequency

Of our 140 cases only 16 Rota +ve patients (12.5%) showed G- typing (Fig. 1) and all of them were of G3 type, while 43 patients (33.6%) showed P typing (Fig. 2) and also all of them were P8. There was statistically significant difference between G3 and nontypable genotyping in degree of dehydration, weight, Vesikari score of severity,

diarrheal duration, vomiting duration and numbers. And there was no statistically significant difference in other factors (Tables 5 and 6). There was statistically significant difference between P8 and nontypable strain in degree of dehydration, Vesikari score of severity, vomiting duration and numbers. But not in weight, number of motions, duration of diarrhea or hospital stay (tables 7 and 8).

Table (5): Concordance between dehydration degree and genotyping G3 in the studied group

Dehydration degree	G3		Nontypable		P-value
	F (16)	%	F (112)	%	
Moderate dehydration (18)	8	50	10	8.9	**0.001**
Severe dehydration (110)	8	50	102	91.1	
Normal (27)	8	50	19	17	**<0.003**
Underweight (101)	8	50	93	83	

F=frequency

Table (6): Comparing clinical data between G3 and nontypable genotyping in the studied group

Variable	G3 (16)	Nontypable (112)	P-value
	Mean ± SD	Mean ± SD	
Veslkari score of Severity	11±1.6	12.1±1.2	**<0.002**
Motion numbers	5.5±1.1	5.7±1.2	0.5
Diarrheal duration (days)	2±0.7	3±0.8	**0.001**
Vomiting duration(days)	1.5±0.4	2.1±0.6	**0.001**
Vomiting numbers	3±0.7	3.7±0.7	**<0.001**
Hospital stay(days)	3.5±1.1	3.4±1.1	0.9

Table (7): Concordance between dehydration degree and P8 strain in the studied group

Dehydration degree	P8		Nontypable (85)		P-value
	(43)	%	%		
Moderate dehydration (18)	00	0.0	18	21.2	**<0.001**
Severe dehydration (110)	43	100	67	78.8	
Normal weight (27)	12	27.9	15	17.6	0.2
Underweight (101)	31	72.1	70	82.4	

F=frequency

Table (8): Comparing clinical data between P8 and nontypable strain in the studied group

Variable	P8 (43)	Nontypable (85)	P-value
	Mean ± SD	Mean ± SD	
Vesikari score of Severity	12.2±1.1	10.1±0.9	**<0.001**
Motion numbers	5.7±1.3	5.6±1.1	0.6
Diarrheal duration (days)	2.8±0.7	2.9±0.8	0.9
Vomiting duration (days)	2.4±0.5	1.8±0.6	**0.001**
Vomiting numbers	3.8±0.9	3.5±0.6	**<0.03**
Hospital stay (days)	3.5±1.3	3.4±1.3	0.9

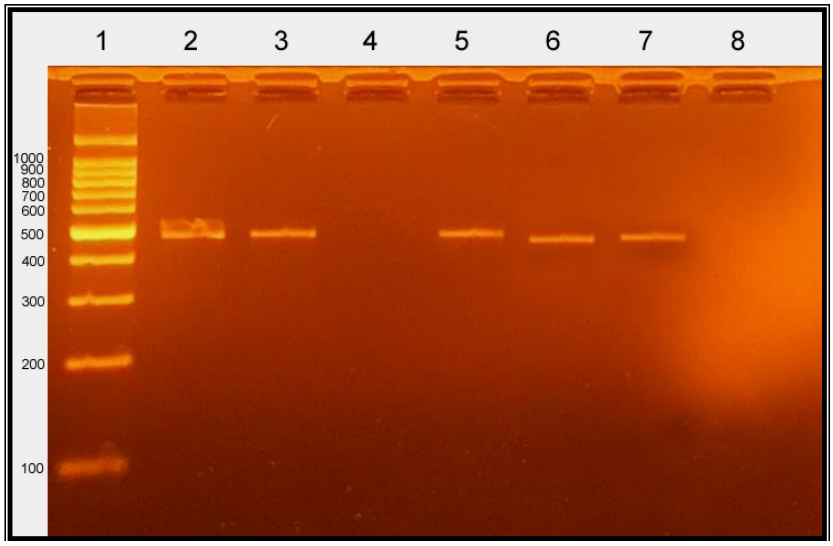

Fig. (1): G genotyping

The gel electrophoresis photo showing:

- In lane 1: The DNA marker (100bp)
- In lanes 2, 3, 5, 6, 7: The bands of G genotypes with its size (500bp)→G3 genotyping
- But in lane 4,8→ No genotype bands appear

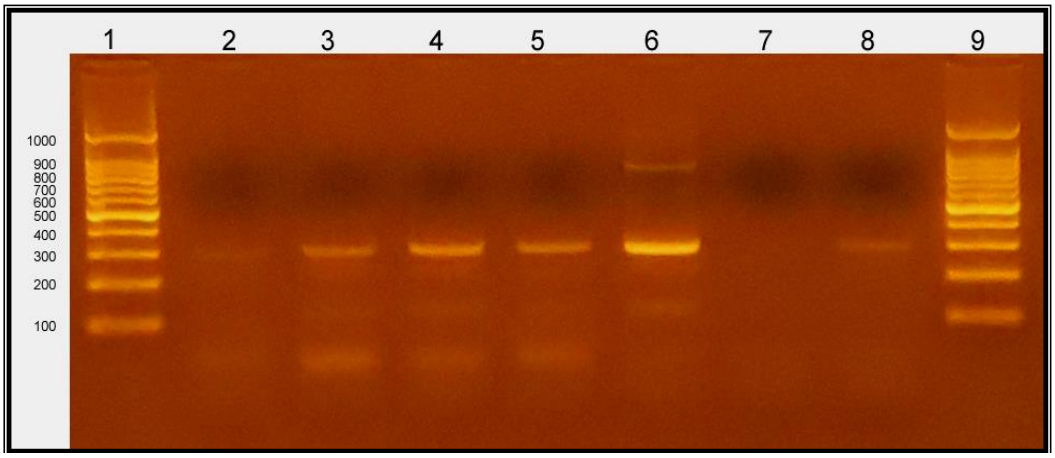

Fig. (2): P genotyping

The gel electrophoresis photo showing:

- In lane 1,9: The DNA marker (100bp)
- In lane 2,3,4 ,5,6,8 : The bands of P genotypes with its size (350bp)→ P8 genotyping
- But in lane 7→ No genotype bands appear.

DISCUSSION

In our study the sample consisted of 140 patients with age range from 6 month to 50 months of them 62.1 were boys and 37.9 were girls. All cases showed moderate to severe diarrheal disorder according to Vesikari scoring system. We found significant difference between moderately and severely dehydrated children regarding their different age group as water represent more of body weight in younger age group, which was in agreement with **Trojnar *et al.*** [12] who reported the significant difference in age and weight with dehydrated children and also in agreement with **Ahmed *et al.*** [13] who reported that most cases of G.E. were in the first year of life, but this finding was in the contrary to **Hegazi *et al.*** [14] who found most cases of rotavirus infection were in the second year of life. This difference may be due to the fact that his study population were vaccinated with rotavirus vaccine.

About 91.4% of the studied group was positive for rotavirus by dipstick. These data are not near to that published by **Ahmed *et al.*** [13] who found that the

prevalence of rotavirus infection among Egyptian children was 40%. This difference may be related to the different study design; as his study was public or epidemiological prospective study but ours was cross sectional and dealt only with hospitalized children with the severest form of infection, which mainly related to rotavirus rather than to other agents [13]. There was no significant difference between rotavirus positive and negative cases regarding both age and sex and this is matched with most published data like that published by **Ahmed *et al.* [13], Bulut *et al.* [15] and Mchaile *et al.* [16]**. But in rotavirus positive cases boys were more affected (63.3%). This was in agreement with **Burton *et al.* [17]** who reported that 71.9% of his study group were males.

We found that the prevalence of rotavirus infection is more during first year of life (35.7% of cases) and decrease subsequently during the following years. These data are matched with that published by **Mchaile *et al.* [16]**, who found that the overall prevalence of rotavirus in this study was 26.4% (73/277) and it was 29/73 (39.7%) in infants aged less than 12 months, 34.2% (25/73) in children aged 13-24 months and 21.9% (16/73) among children older than 24 months.

We found that, there was significant difference between rotavirus positive and negative cases in Vesikari score of severity, duration of hospital stay and weight of patients, which was in agreement with **Bass *et al.* [18]** But there was no significant difference regarding dehydration and this was in concordance with study reported by **Paul *et al.* [19]** and in agreement with **Karyana *et al.* [20]** who reported that clinical manifestations of rotavirus were more severe than those of other causes of viral gastroenteritis and also in agreement with **Hegazi *et al.* [14]** who found marked significant difference regarding the G.E. severity between positive and negative rotavirus cases.

Our study revealed that 76.4% of the studied group were under weight and 87.1% of them had severe dehydration while **Sudarmo *et al.* [21]** reported that 56.9% had normal weight. This may be due to the effect of dehydration on the body weight and partially due to the case selection as our cases were those who needed hospitalization.

Regarding the genotyping of VP7 in the present study, the only detected was G3 genotype and represented 12.5% of the studied group. This is in agreement with **Magzoub *et al.* [22]** who found that only G1 (83.3%) and G9 (16.7%) were detected in his population and it was in disagreement with **Tate *et al.* [23]** who reported that 53% had G3 strains and **Walker *et al.* [24]** showed that G1 and G9 genotypes were the most prevalent. This difference may be due to different laboratory protocols and resources. And it is also in disagreement with **Ahmed *et al.* [13]** who reported that the most common was G2, G1 and G9 respectively.

We also found that 33.6% of the studied group had P8 strain. This was in agreement with **Trimis *et al.* [25]** who reported that 24.7% had P8 and also in agreement with **Magzoub *et al.* [22]** who also detected only P8 in one case of his 121 cohort and our data also were in agreement with **Bulut *et al.* [15]** who found P8 as the most prevalent type. While **Soenarto *et al.* [26]** showed that 55.6% had P6 strain and 17.5% had P8 strain and **Ahmed *et al.* [13]** were able to detect only 58% of his population while 42% were nontypable for P strains.

In the present study 53.9% of the studied group were nontypable G nontypable P, which was in disagreement with **Sprengers *et al.* [27]** who reported that only 1.6% of his cases were nontypable.

The present study showed that there was statistically significant difference between G3 strain and nontypable G strains regarding dehydration and Vesikari score of G.E. severity with more severe in nontypable G, which was in agreement with **Sudarmo *et al.* [21] a**nd **Lopman *et al.* [28]** who reported a significant difference in several analysis.

There was statistically significant difference between P8 and nontypable G strain in Vesikari score of severity, vomiting duration, number of vomitus, degree of dehydration and weight but there was no significant difference in other factors, which was in agreement with **Rudan *et al.* [29] and Neves *et al.* [30],** while **Clemens *et al.* [31]** reported that P strain and nontypable G showed no significant difference in total Vesikari score of severity [29-31].

Conclusion:

- Rotavirus still represent high percentage of hospitalized cases of G.E. in Zagazig University Hospital.
- Most cases of severe G.E and complicated cases are related to rotavirus infection
- There is significant difference in severity and complications between positive and negative rota cases.
- Rotavirus G.E represents hard financial burden in our community.

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