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Genotyping of Rotavirus RNA by Sequencing among Children with Diarrhea at Zagazig University Hospital

Elhamy Rifky abdelkhalik[1], Mohamed Abdelkader Adallah Almalky[1], Rania Mohamed Mohamed Ebrahim Amer[2], Hazem Ali Abd Elraouf[1]
Departments of [1]Pediatrics and [2]Microbiology and Immunology,
Faculty of Medicine, Zagazig University, Sharkia, Egypt
***Corresponding author:** Hazem Ali Abd Elraouf, **Mobile:** (+20) 01003497579, **E-Mail:** hazem032012@gmail.com

ABSTRACT

Background: Worldwide, rotavirus (RV) is the most common cause of severe gastroenteritis among infants and young children. While diarrhea is the second most common cause of fatal childhood illness. RV can be detected in high concentrations in the stool of children suffering from gastroenteritis.

Objectives: To determine rotavirus genotyping among diarrheic children at Sharkia Governorate.

Patients and Methods: For one-year surveillance, the data and the stool samples were gathered from January 2015 to January 2016. A total of 140 stool specimens were collected from the inpatients diagnosed with acute diarrhea in the Pediatric Department, Zagazig University Hospitals.

Results: Our results showed that there was a statistically significant difference between positive and negative rotavirus infection cases in Veslkari score of severity and hospital stay. 12.5% of the study group had G3 genotype and 33.6% of the study group had strain P8. By comparing clinical data between G3 and non-typeable genotyping in the studied group, there was a statistically significant difference between G3 and non-typeable genotyping in the Veslkari score of severity. There was a highly statistically significant negative correlation between the Veslkari score of severity and weight. On the other side, there was a statistically significant positive correlation between the Veslkari score of severity and age.

Conclusion: We concluded that rotavirus represents a high percentage of hospitalized cases of GE in Zagazig University Hospital with a significant difference in severity and complications between positive and negative rota cases.

Keywords: Rotavirus RNA, Genotyping, Sequencing, Children, Diarrhea.

INTRODUCTION

The most common cause of severe gastroenteritis among infants and young children is Rotavirus infection [(1)]. Diarrhea is the second most common cause of fatal childhood illness, about 1.34 million deaths occur around the world among children aged less than 5 years due to RV [(2)]. 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 [(3)].

At least one episode of RV gastroenteritis encounters every child 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 [(4)].

RV can be detected in high concentrations in the stool of children suffering from gastroenteritis. Control measures such as improved sanitation are not effective in preventing this disease [(5)]. Rotavirus (RV) belongs to the Reoviridae virus family and the virion comprises three concentric protein layers**.** 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**.** Out of the 12 G and 15 genotypes known to infect humans, genotypes G1P [81, G2Pl4], G3P [8], G4P [8], and G9P [8] cause over 90%of rotavirus diseases worldwide [(6)]**.**

Objectives: To determine rotavirus genotyping among diarrheic children at Sharkia Governorate.

PATIENTS AND METHODS

This cross-sectional study was conducted in the Departments of Pediatrics and Medical Microbiology and Immunology as well as the Medical Scientific and Research Centre, Faculty of Medicine, Zagazig University Hospitals.

Sample size estimation:

For one year of surveillance, the data and the stool samples were collected from January 2015 to January 2016 (in November, December, and January months which was the peak of RV). A total of 140 stool specimens were collected from the diagnosed inpatients with acute diarrhea in the Pediatric Department, Zagazig University Hospitals.

Inclusion criteria: Infants and children aged from one month to five years presented with gastroenteritis or acute diarrhea.

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

Data Collection:

1. **All study subjects underwent:** Thorough history taking including personal history (age, sex,

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name, number of children, education, and occupation of the parents) and present history (symptoms, duration of symptoms, temperature of the child, hydration state).

2. **Physical examination.**

3. **Laboratory investigations include** Ag detection of rotavirus (by Immunochromatography), and genotyping for (Ag positive samples) by PCR technique.

Materials:

1. Sterile cup for stool sample collection.
2. Falcon tubes (15 ml) for the preservation of stool samples at (-70°c) deep freezing for genotyping.
3. RIDA® Quick rotavirus kit: which is a rapid *in vitro* immunochromatographic test for the qualitative determination of rotaviruses in stool samples.
4. QIAamp Viral RNA mini kit (Qiagen); (Extraction kit for viral RNA).

Methods:

- ***Sample collection:*** Samples were collected as watery diarrhea in a sterile container (Eppendorf safe-lock tube) and refrigerated at (2-8ºC) up to 72 hours from the collection and each sample was divided into two tubes one frozen at –20ºC for immunochromatography test and the other at-80ºC for PCR genotyping.

- **RIDA® Quick test:**

A) Procedure:

The reagents were bought at room temperature(20 -25ºc). One ml was pipetted of the extraction buffer (diluent) into a test tube. One hundred µl or 50 mg stool sample was added to the diluent. The sample was homogenized on a vortex mixer or by suction and ejection of the stool suspension using the disposable pipette provided. The stool sample was allowed to settle for three minutes then 0.5ml of the clear supernatant was pipetted into another test tube. The strip for the test was removed from the box and immersed in the collected supernatant up to the arrow mark at most.

B) Interpretation

- **Positive:** A red test band along with the blue control band. The color intensity varies and is dependent on the quantity of antigens in the sample.
- **Negative:** Only the blue control band appears.
- **Not valid:** No blue control band. In this case, the test must be repeated with a new strip.

Other band colorations and discolorations which appear after 10 minutes have no diagnostic value. These phenomena may be the result of using too much stool sample.

Viral RNA extraction by QIAamp viral RNA mini kit (According to the manufacturer's instructions).

Genotyping: For genotyping of rotavirus 2 types of genotyping were done (genotyping for Pproteins{Pgenotyping}and genotyping for G proteins{G genotyping}) [According to the WHO protocol [7]].

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical Analysis:

All data were collected, tabulated, and statistically analyzed using SPSS version 22. Continuous Quantitative variables were expressed as the mean±SD & range, and categorical qualitative variables were expressed as absolute frequencies (number) & relative frequencies (percentage). A T-test was used to compare two groups of normally distributed data. Categorical data were compared using the Chi-square test ($\chi 2$ test). Pearson's correlation test was used to detect the closeness of association between quantitative variables. All tests were two-sided. p-value< 0.05 was considered statistically significant (S), p-value < 0.001 was considered highly statistically significant (HS), and p-value ≥ 0.05 was considered statistically insignificant (NS).

RESULTS

This table shows that our sample consisted of (140) patients with age ranges from (1-50) months and 62.1% of them are males. the clinical criteria with Veslkari score of severity ranged from (9-15, diarrheal duration ranged from 1 to 5 days, vomiting continued from 1 to 3 days and hospital stay ranged from 2 to 6 days. (91.4%) of the study group were positive Rota by dipstick **(Table 1)**.

Table (1): Demographic and clinical data as well as the prevalence of Rotavirus among the studied group

Demographic data	**Mean ± SD Median (Range)**	
Age (months)	20.7±12.2 19 (6-50)	
Variable	**F (140)**	**%**
Age group		
1-12	50	35.7
12-24	38	27.1
24-36	36	25.7
36-50	16	11.4
Sex		
Male	87	62.1
Female	53	37.9
Clinical data	**Mean ± SD median (Range)**	
Veslkari score of Severity	11.9±1.3 12 (9-15)	
Diarrheal duration (days)	2.8±0.8 3 (1-5)	
Vomiting duration (days)	2.1±0.6 2 (1-3)	
Hospital stay (days)	3.4±1.3 3 (2-6)	
Dipstick	**F (140)**	**%**
Negative	12	8.6
Positive	128	91.4

This table shows that (12.5%) of the study group had a G3genotype. (33.6%) of the study group had strain P8 **(Table 2)**.

Table (2): Genotyping and prevalence of strain P in the studied group:

Variable	**F (128)**	**%**
Genotyping		
G3	16	12.5
Non-typable	112	87.5
Strain P		
P8	43	33.6
Non-typable	85	66.4

This table shows that there was a statistically significant difference between positive and negative in G3. But regarding strain P, there was no statistically significant difference between positive and negative **(Table 3)**.

Table (3): Comparing genotyping and strain P between positive and negative Rotavirus in the studied group

Variable	**Positive F (128) %**	**Negative F(12) %**	**χ^2**	**p-value**
Genotyping				
G3 Non-typable	16 12.5 112 87.5	00 0.0 12 100.0	FET	**0.01***
Strain P				
P8 Non-typable	43 33.6 85 66.4	00 0.0 12 100.0	FET	0.3

*Statistically significant difference ($P \leq 0.05$)

This table shows that there was a statistically significant difference between moderate and severely dehydrated children in different age groups as water represents more body weight in younger age groups **(Table 4)**.

Table (4): Comparing dehydration degrees in different age groups in the studied group:

Age group	Moderate dehydration F (18) %	Severe dehydration F(122) %	χ^2	p-value
6-12 months (50)	14 77.8	36 29.5	18.2	**0.001****
12-24 months (38)	00 0.00	38 31.1		
24-36 months (36)	4 22.2	32 26.2		
36-50 months (16)	00 0.00	16 13.1		

** Statistically highly significant difference ($P \leq 0.001$)

This table shows that there was a statistically significant difference between G3 and non-typeable genotyping in the degree of dehydration **(Table 5)**.

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

Dehydration degree	G3 F (16) %	Non-typable F(112) %	χ^2	p-value
Moderate dehydration(18)	8 50	10 8.9	19.5	**0.001****
Severe dehydration (110)	8 50	102 91.1		

** Statistically highly significant difference ($P \leq 0.001$)

This table shows that there was a statistically significant difference between P8 and non-typeable strain in the degree of dehydration. There was a statistically significant difference between P8 and non-typeable strain in weight **(Table 6)**.

Table (6): Concordance between (dehydration degree, weight) and P8 strain in the studied group:

Dehydration degree	P8 F (43) %	Non-typable F(85) %	χ^2	p-value
Moderate dehydration (18)	00 0.0	18 21.2	10.5	**0.001****
Severe dehydration(110)	43 100	67 78.8		
Weight	**P8 F (43) %**	**Non-typable F(85) %**	**χ^2**	**p-value**
Normal (27)	12 27.9	15 17.6	1.8	0.2
Underweight (101)	31 72.1	70 82.4		

This table shows that there was a highly statistically significant negative correlation between the Veslkari score of severity and weight. On the other side, there was a statistically significant positive correlation between the Veslkari score of severity and age **Table (7)**.

Table (7): Correlation between Veslkari score of severity with age and weight of the studied group:

Variable	Veslkari score of severity		
	r	p	SIG
Age (months)	0.2	0.004*	S
Weight	-0. 4	0.001**	HS

*Statistically significant difference ($P \leq 0.05$)

** Statistically highly significant difference ($P \leq 0.001$)

DISCUSSION

We found that all cases showed moderate to severe diarrheal disorder Also we found in our study statistically significant difference between moderate and severely dehydrated children in different age groups as water represent more of body weight in the younger age group which was in agreement with **Ahmed *et al.*** [8] who reported most cases of G.E. were in the first year of life.

The studied group was positive Rota by dipstick with a percentage of 91.4%. This data is not near to that published by **Ahmed *et al.*** [8] who found that the prevalence of rotavirus infection among Egyptian children was 40% this difference may be related to the different design as his study was a public or epidemiological prospective study but ours was cross-sectional and deal only with hospitalized children only with the severest form of infection which mainly related to rotavirus rather than to other agents. There is no significant difference between positive and negative cases regarding both age and sex and this is matched by **Bulut *et al.*** [9]. But in positive cases, boys were more affected by about 63.3% this was in agreement with

Burton *et al.* [10] who reported that 71.9% of their study group were males.

We found that the prevalence of rotavirus infection is more during the first year of life (35.7%) of cases and decreases subsequently during the following years. This data is matched with that published by **Mchaile *et al.* [11],** who found The overall prevalence of Rotavirus in this study was 26.4% (73/277); 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.

The present study showed vesikari score of severity ranged from (9-15) and diarrheal duration ranged from (1-5) days with several motions ranging from (4-8/day) and vomiting continued from (1-3) days. And we found in our study there was a significant difference between positive and negative rotavirus in vesikari score of severity and hospital stay which was in agreement with **Bass *et al.* [12].** This confirms the findings by most authors which stated that RV gastroenteritis is more severe with more severe complications than other types of gastroenteritis.

In our study, there was a statistically significant difference between positive and negative rotavirus in weight but regarding dehydration, there was non-significance that was in concordance with the study reported by **Paul *et al.* [13].** This was in agreement with **Karyana *et al.* [14]** 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.* [15]** who found a marked significant difference regarding the G.E. severity between positive and negative rotavirus cases.

This was in agreement with **Karyana *et al.* [14]** 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.* [15]** who found a 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 underweight and 87.1% of them had severe dehydration while **Sudarmo *et al.* [16]** reported that 56.9% were 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 that who needed hospitalization.

Regarding the genotyping of VP7 in the present study, the only detected was G3 genotype representing 12.5% of the studied group this is in agreement with **Magzoub *et al.* [17]** 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.*, [18]** who reported that 53% had G3 strains and **Walker *et al.* [19]** showed that G1&G9 genotypes were the most prevalent. This difference may be due to different laboratory protocols and resources. And also in disagreement with **Ahmed *et al.* [8]** who reported that the most common was G2, G1, and G9 respectively.

In the present study, 53.9% of the studied group were non-typeable G and non-typeable P which was in disagreement with **Sprengers *et al.* [20]** who reported that 1.6% of them were non typable.

The present study showed that there was a statistically significant difference between G3 strain and non-typeable G strains regarding dehydration and Vesikari score of G.E. severity with more severe in non-typeable G this was in agreement with **Lopman *et al.* [21]** who reported the significant difference in analysis.

In the present study, there was a statistically significant difference between P8 & non-typeable G strain in the degree of dehydration and weight that was in agreement with **Neves *et al.* [22].**

There was a statistically significant difference between P8 & non-typeable G strain in vesikari score of severity regarding vomiting duration and number of vomitus otherwise there was no significance in other factors that were in agreement with **Rudan *et al.* [23].**

Also, we found a highly statistically significant negative correlation between the vesikari score of severity and weight that was in agreement with **Schnadower *et al.* [24].** On the other side, there was a statistically significant positive correlation between the vesikari score of severity with age which was in agreement with **Steele *et al.* [25].**

CONCLUSION

We concluded that Rotavirus represents a high percentage of hospitalized cases of GE in Zagazig University Hospital with a significant difference in severity and complications between positive and negative rota cases.

Conflict of interest: The authors declare no conflict of interest.

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Author contribution: Authors contributed equally to the study.

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