Molecular Detection of Rotavirus in Infants Suffering from Acute Gastroenteritis in Al-Muthanna Governorate Iraq

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Abstract
Background: Rotavirus is the main infective agent of acute gastroenteritis (AGE) in children under the age of two years and causing significant morbidity as well as mortality throughout the world. Objective: The purpose of this study is to determine the frequency of the one of the most important enteric viruses (Rotavirus) in stool specimens of children aged less than 2 years. The age, sex distribution, type of feeding and the area of residence were noted. Methods: A total of 50 fecal specimens (including 28 males and 22 females) were collected from infants under 2 years of age in Al_ Muthanna Governorate in South of Iraq during four months (from November 2021 to March 2022). The samples were classified into four groups according to the age of the children: ≤5 months, 6-10 months, 11-15months and 16-20 months. Rotavirus was detected by using RT_PCR technology. Results: The results of this study revealed that Rotavirus were the main cause of diarrhea for children which about 76%. The results showed that the number of male children diarrhea patients was more than their female. The number of children infected with diarrhea, caused by Rotavirus was high (16) in age group ≤5 months. The infected children with Rotavirus were classified according to their feeding; natural feeding, mixed feeding and artifical feeding. The number and ratio of infected children with Rotavirus were 2 (5.3%), 17 (44.7%) and 19 (50%) respectively. Conclusions: It is concluded from this study that human Rotavirus is the main causative of acute gastroenteritis in infants under 2 years old in AL.Muthanna Governorate. It may cause sporadic infection in closely groups of children and this should be considered while treating acute gastroenteritis patients
Key words: Acute gastroenteritis, Human Rotavirus , Real Time – PCR

1. Introduction

Viruses, parasites, and bacteria are among the most common infectious agents causing acute gastroenteritis in children. Amongst them, viruses such as Rotavirus and Norovirus contribute to the greatest rate of infection [(1)]. Rotaviruses are the most common agent causing AGE in children worldwide [(2)].They are responsible for most cases of gastroenteritis and mortality in infants and children below 5 years of age, especially in developing countries [(3)]. Approximately 200,000 people die annually from infection. Although the infection rate has decreased with the discovery of vaccines, infections are still more common throughout the world [(4)].

Early symptoms of the infection with RV include fever, diarrhea, and vomiting . Additionally, RV can lead to death, most often in poorly and low-income countries, due to dehydration and lack of medical care [(5)].The Rotavirus spreads fastly from the unhealthy to healthy. The virus transmits through a fecal-oral life cycle [(6)].The virus is held in the feces as it exits the body and it istransmit by exposure and contact with a sick individual. The virus then enters the body through the oral passageway, and this can occur by several ways. [(7)].The incubation period of infection is usually 12–72 hours [(8)]. Electrolyte imbalance and dehydration are the most common complications. If the dehydration is severe enough, it can lead to shock, coma and even death. Children with malnutrition status are at increased risk [(9)].Rotaviruses are non-enveloped double-stranded RNA (dsRNA) viruses that have a complex architecture of three concentric capsids that surround a genome of 11 segments of dsRNA .The segments of RNA encode 6 structural viral proteins (VP1, VP2, VP3, VP4, VP6 and VP7) and six non-structural proteins (NSP1, NSP2, NSP3, NSP4, NSP5 and NSP6) [(10)]. Ten different rotavirus species (A–J) have been classified according to sequence and antigenic differences of VP6 [(11)].

2. Materials and Methods

2.1. Study Population and Sample Collection

A total of 100 blood and stool specimens were collected between November 2021 to April 2022 from infants less than 2 years of age who were admitted with acute gastroenteritis to the Women and Children Hospital, Iraqi Samawa. Demographic and clinical data were collected through conducting a questionnaire. Stool samples were collected in viral tansport media (VTM) while blood in EDTA tubes until use and extraction then transfer at -80 C° in the freeze. All specimens were transported to the laboratory for processing and investigations.

2.2. Extraction of Viral Nucleic Acid from Clinical Specimens

By using specific viral DNA/RNA extraction kit (Intron/Korea); the viral genomic was extracted and purifying from the stool and blood of infants patients

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with acute gastroenteritis infection as a first step to amplify the target Rotavirus-RNA. The viral genomic was converted to cDNA, and after completing the conversion of all the RNA to cDNA, the examination was carried out using nanodrop technique.

2.3. Viral Genome Conversion to cDNA

The extracted viral genome is converted to cDNA according to the steps used by manufacturer company (biosharp / China).

2.4. Detection of Rotavirus infection by Real Time – PCR

RT-PCR technique was performed on isolates in method that used for Rotavirus genotyping by using DNA sequencing analysis. The detection was applied according to the procedure that consists of the mixture described in the table (2-1) , and the RT.PCR conditions was in terms of temperatures and the number of cycles as described in the table (2-2).

Table (2-1) PCR Reaction Mixture

Volume	Composition
10 µℓ	2X Real-Time Smart mix
1 µℓ	Primer F (10 pmole\ µℓ)
1 µℓ	Primer R (10 pmole\ µℓ)
2 µℓ	Template DNA
6 µℓ	Add D.W.

Table (2-2) PCR Condition

Cycles	Time	Temperature
1	15 min	95°C
30	20 sec	95°C
30	40 sec	55°C
30	30 sec	72°C

3. Results

3.1 Rotavirus infection and results of RT. PCR

One handred stool specimens involved in this study 50 were AGEI patients and 50 healthy control. This study used RT-PCR to evaluate the presence of human Rorovirus in stool samples of infants with acute gastroenteritis who have attended to Children and Women Hospital in Samawa city and other hospitals in Al-Muthana Governorate. The positive result was 76% (38 out of 50 cases) as positive and 24% (12 out of 50 cases) as negative. While the control samples were 100 per cent negative after detection by the same technique (RT-PCR),

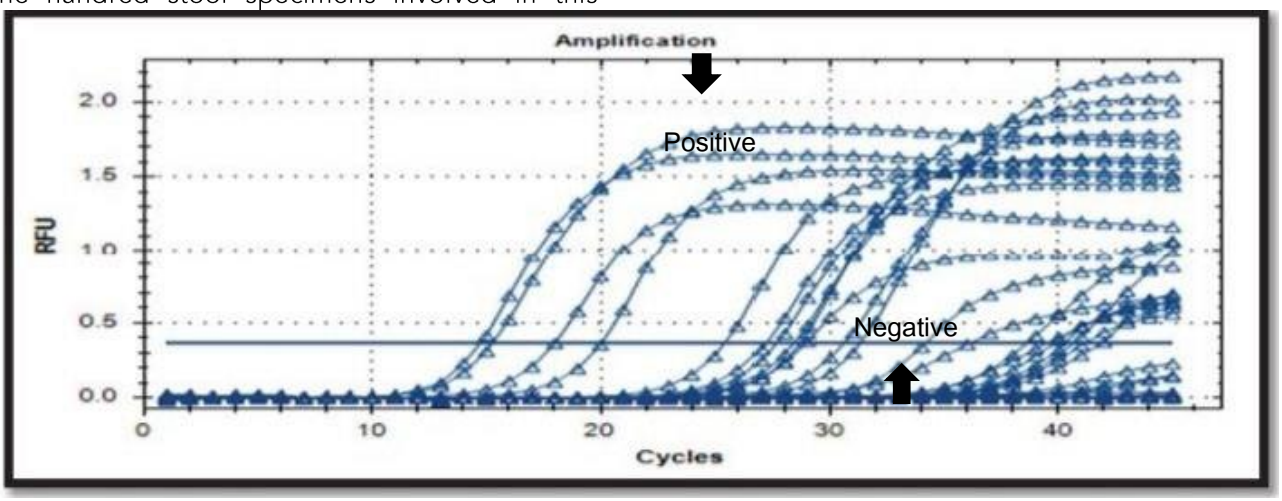

Figure 1: The positive samples of Rotavirus by RT-PCR

3.2 Sex Distribution of Rotavirus-Positive in Diarrheal Group of Infants:

According to diarrhea suffered infants showing that 76% were caused by Rotavirus. Table (1) demonstrate of Rotavirus-positive in diarrheal group of children based on sex. The study found that from 38 children with diarrhea caused by Rotavirus, the percentage of male (57.89%) was higher than female (42.11%). The difference between male and female was highly significant (P < 0.01).

Table (3-1): Sex Distribution of Rotavirus-Positive in Diarrheal Group of Infants

Gender	Rotavirus			
	Positive	%	Negative	%
Male	22	57.89	6	50
Female	16	42.11	6	50
Total	38	100	12	100
Chi-square = 10.268, df = 1, chi = 6.635, significant (P<0.01)				

3.3 Distribution of Patients with Rotavirus According to the Feeding Type:

Table (2) showed the distribution of patients with Rotavirus based on the feeding types. These types were artificial, mixed and natural feeding. The number and percentage of patients according to their feeding were 19(50%), 17(44.7%) and 2(5.3%)

respectively.The differences among three types of feeding were highly significant (p < 0,01).

Table (3-2): Distribution of Patients with Rotavirus According to the Feeding Type				
Feeding	Rotavirus			
	Positive	%	Negative	%
Artificial	19	50	3	25
Mixed	17	44.7	5	41. 7
Natural	2	5.3	4	33.3
Total	38	100	12	100
Chi-square = 12.036, df = 2, chi = 9.210, significant (P<0.01)				

3.4 Age Distribution of Rotavirus-Positive in Diarrheal Group of Infants:

Table (3) showed the number and percentage of patients infected with Rotavirus according to age groups. The maximum number 16(42.1%) was in age group ≤5 months. Whereas the minimum number 5(13.1%) was in age group (16-20) months. The difference among age groups was highly significant.

Table (3-3): Age Distribution of Rotavirus-Positive in Diarrheal Group of Infants				
Age groupsMonths	Rotavirus			
	Positive	%	Negative	%
≤5	16	42.11	3	25
6-10	10	26.31	4	3.33
11-15	7	18.42	4	3.33
16-20	5	13.16	1	8.34
Total	38	100	12	100
Chi-square = 14.27, df = 4, chi = 2.59, significant (P<0.01)				

3.5 Distribution of Rotavirus in Diarrheal Group of Infants According to the living style:

The distribution of patients with Rotavirus according to the living style was explaned in Table (4). The highest number of patients 28(73.7%) lived in rural area. However, the lowest number of patients 10(26.3%) lived in urban area. The differences between them was highly significant (p < 0,01).

Table (3-4): Distribution of Rotavirus in Diarrheal Group of Infants According to the living style:				
ResidenceType	Rotavirus			
	Positive	%	Negative	%
Urban	10	26.32	6	50
Rural	28	73.68	6	50
Total	38	100	12	100
Chi-square = 15.326, df = 1, chi = 13.277, significant (P<0.01)				

4. Discussion

This study was performed to evaluate the prevalence of Rotavirus, among the infants with acute gastroenteritis in Al Muthanna Governorate, and the association of Rotavirus and different factors including sex, age, feeding type in infants under2 years of age and type of residence area (urban or rural).

The prevalence of Rotavirus in the present study for male 22 (57.89%) was higher than females 16 (42.11%) patients.This study agreed with the result of study conducted by Mohammed et al. (2020) [12] which revealed that A higher rate of Rotavirus infection was detected in males more than females. Also the recent results concur with another study of Moyo *et al.*,(2014) [13] who found male infected with Rotavirus was (61.2%) higher than in female was (38.8). The reason for the sex difference is not determined, but maybe implying that males have a more stool excretion rate of Rotavirus infection and are more susceptible to infection . This distinction sex susceptibility is uncertain and needs further investigation [14].

The results of this study revealed that are significant differences between the age groups and the percentage of Rotavirus-positive patients with highest value is (42.11%) 16 cases at age group of (≤5) months, and the lowest one is (13.16%) 5 cases at age group of (16-20) months. the results of this study are consistent with a study conducted by Chissaque *et al.*,(2021)[15], which indicates that infants aged (0-11) months are more likely to be infected compared to children aged (24-60) months, possibly due to malnutrition or improper and unsuitable nutrition healthy. While, this result is not agreed with the data obtained in Iran by Nasab *et al.*,(2016)[16] which showed that the highest value of positive rotavirus in infants was 46.9% between (13-24) months, whereas the lowest value was (6.1%) at age group of (37-60) months.

The weaknesses of immune system of infants, in addition to the lack of health awareness and personal hygiene for some mothers or primary health care staff, have an important role in the occurrence of Rotavirus infection.

During this study, the relationship between the incidence of Rotavirus and the type of feeding shows the rate of rotavirus gastroenteritis is highest in infants who used artificial feeding (bottle feeding) with 19 (50%) children, followed by mixed feeding and natural feeding with 17 (44.74%) and 2 (5.26%), respectively. This variation of the above results is statistically significant (p<0,01).It is consistent with another local study conducted by Ali et al., (2010)[17] which found that Rotavirus gastroenteritis is higher in infants with bottle

feeding. The results of another study by Nakawesi *et al.*, (2010)[18] showed that children who are fed artificially (bottle feeding) were more susceptible to diarrhea by Rotavirus than those who were breastfed or mixed. This is due to the ease of transmission of viruses in contaminated feeding bottles.

This study discussed the relationship between gastroenteritis with rotavirus in children under 2 years of age with residence (urban and rural), as it showed that the incidence of rotavirus diarrhea in rural areas is 28 (73,68%) higher than in urban areas which was 10 cases (26,32%).

These results are consistent with the study conducted by Ali *et al.* (2010)[17] in Kirkuk, where the number of positive cases in urban areas was 12 cases, while in rural areas it was 15 cases. On the other hand, it does not match the findings of Suresh et al., (2020)[19] in western Rajasthan, in which the number of positive cases was more in urban areas than in rural areas.

Conclusion

The infection rate of human Rorovirus among infant under 2 years old suffering from acute gastroenteritis in Al-Muthanna Governorate during the period of this study was 76%. , The highest rate (68%) was recorded among males and females within age group less 1 year. The whole positive results were from different places in Al-Muthanna .The result of Real time polymerase chain reaction demonstrates that 38 positive cases human Rorovirus from 50 cases infected with AGEI . Human Rorovirus may cause sporadic infection in closely groups of people and this should be considered while treating acute gastroenteritis patients.

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