

DETECTION OF ROTAVIRUS TYPE A BY REVERSE TRANSCRIPTASE PCR IN CHILDREN SUFFERING FROM VIRAL GASTROENTERITIS IN BABYLON PROVINCE

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Abstract:

Rotavirus type A is one of the viruses that cause viral gastroenteritis. This research was done to detection Rotavirus type A in watery stool samples of children suffering from viral gastroenteritis. Stool samples were collected in four months from November 2019 to March 2020 from three hospitals in Babylon province of Iraq are Hashemite Hospital was collected 50 stool specimens, while Al-Noor Hospital for Maternity and General Al-Qasim Hospital were collected 25 stool specimens. A total of 46 males and 29 females were diagnosed with viral gastroenteritis. Two methods used in the present study for detection Rotavirus and Rotavirus type A were rapid test and reverse transcriptase PCR . The results of rapid test were indicated to 26 positive samples for rotavirus out of 75 stool samples while the results of reverse transcriptase PCR for detection Rotavirus type A were indicated to 6 positive samples out of the 26 stool samples.

Keywords: *Rotavirus Type A, Rapid Test, RT-PCR.*

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Introduction:

One of the signs of inflammation of the intestines, which can be caused by a variety of bacteria, viruses, and parasites [1, 2], is diarrhea. In impoverished nations, babies and young children are particularly vulnerable to the diarrheal diseases that can lead to morbidity and mortality. [1]. Rotavirus is responsible for more than 600,000 annual deaths in children under the age of five, resulting in 25 million medical visits, two million hospitalizations, and two million lost days of school [2]. One of the viruses that can cause viral gastroenteritis in infants and young children, as well as in young animals of a variety of species [3], rotavirus can also affect young animals. Infections caused by the rotavirus can be passed on to others and most commonly take place between November and April, throughout the winter and early spring months. Infection with the rotavirus was prevalent in environments with a high concentration of children, such as day care facilities [4].

Rotavirus type A is the most prevalent human infection, and it is transmitted from feces to the mouth via the fecal-oral pathway. The virus is most prevalent in the winter. In the small intestine, they infect mature absorptive enterocytes [5]. The symptoms of these viruses that are most commonly seen in newborns include vomiting, stomach cramps, and diarrhea, and the incubation period for Rotavirus type A is between three and eight days [6]. Oral rehydration is the treatment that is used the most frequently [5,6]. The purpose of this study was to investigate the pathogenesis of Rotavirus type A, which is the virus responsible for diarrhea in infants in Babylon province who are younger than three years old.

The following points were included in the study's objectives:

- 1-Using a rapid test, the Rota virus can be detected.
- 2-Detection of Rota virus type A using a reverse transcriptase polymerase chain reaction based on positive samples obtained via a rapid examination.

MATERIAL AND METHOD

1-Stool Samples Collection

Seventy five stool specimens collected from children suffering from viral gastroenteritis in three hospitals in Babylon Province of Iraq were Al-Noor Hospital for Maternity and General Al-Qasim Hospital General Hashemite Hospital during four Months (From November 2019 to March2020). Sex of patients were included 46 males and 29 females suffering from acute viral gastroenteritis. After the feces specimens were cleaned and centrifuged at 8000 rpm for 15 minutes at 4°C, the supernatants were collected and placed in a freezer at a temperature of -20°C until they were used for nucleic acid extraction.

2- Immunological study

The Rapid test was used in this study for detection Rotavirus in 75 stool specimens (supplied by Biotech. USA) the principle of this test depend on immunological reaction between Antigen and Antibody of the suspected cases of Rotavirus.

3-Molecular study:

3-1- RNA extraction:

RNA extraction from stool positive samples of Rotavirus conducted according to several steps are:

1-The stool samples were centrifuged at 10000 xg for 3minute to removed cell debris then transferred the clear supernatant to 1.5ml microcentrifuge tube

2- Genezol reagent were added at 700ul

3- mixed well by vortex.

4-Incubated for 5 minutes, then vortex another 5 minutes.

5- centrifuged 10000 For two minutes

6 the ethanol was added and filtrate was taken .

7-vortex for solution.

8-put RB column in column collection Transmits to him 700ul From to mixture

9—centrifuged 10000 For two minutes Then it loads supernatant in collection tube Be repeated added mixture in column For a while Running out of quantity

10-removed collection tube and put new collection tube

11- pre-wash Buffer was added at 400ul

12- centrifuged 10000 For two minutes

13- wash Buffer was added at 600 ul

14- centrifuged 10000 For two minutes

15-put column in epindorf added 100 ul from Ranase-free water On for a direct filter centrifuged 10000 For one minute.

3-2- cDNA synthesis

The RNA extraction from positive Rotavirus in stool was convert to cDNA in several steps included :

- Ten μ of extracted nucleic acid (dsRNA) were transferred to a PCR tube and denatured at 97°C for 5 minutes before chilling for 2 minutes on ice.

- The capacity of the RT-mastermix (bioneer) was increased to 20 liters by adding nucleases-free water after five liters of denatured dsRNA and one liter of each Primer VP7-F and VP7-R (20 pmoles/ 1μ) were added to it. The total volume of the mixture was also raised up to 20 liters.

- Incubation of tubes at 37°C for 1 hour, then at 95°C for 5 minutes to avoid the reaction.

- The tubes were chilled for 2 minutes on ice.

- The cDNA that has been synthesized can be used right away or stored at -20°C until required.

Table (1) PCR reaction mixture compositions .

Components	Concentration	Volume
cDNA		5µl
Master mix (promega)	2X	10
VP7-F primer	20 pmoles /µl	1µ
VP7-R primer	20 pmoles /µl	1µ
Nucleases free water		3µ

3-3-Agarose gel electrophoresis:

gel electrophoresis method was performed as follows:

1- preparation of gel (2.5%) was done by dissolving 1.25 gram of agarose in 50 ml of 0.5X TBE buffer and heating for 2 minutes in a microwave oven.

2-The homogenized agarose was cooled in a water bath to 55°C.

3-A 50 µl solution of ethidium bromide stock (1 mg/ml) was applied to the gel and swirled together.

4-The gel poured and let to polymerize for 30 minutes .

5- The gel was transferred to the electrophoresis apparatus and soaked in 0.5 TBE running buffer.

6-A mechanical pipet was used carefully to transferred five microliters of PCR product into the gel wells.

7- Electrophoresis was performed according to 100 Volts and 40 Amperes about 60 minutes.

8- The image was evaluated so that the genotype could be determined.

RESULTS AND DISCUSSION

1-Rapid test for Rota virus detection:

The Rapid test is a quick examination of suspected cases of Rotavirus, .The results of this study recorded 26 stool positive samples for Rotavirus while 49 stool samples are negative . These results reported in table (1)

Table (1) Distribution of stool samples according to etiological agents

No. of stool samples	No. of Bacteria	No. of Parasites	No . of Rotavirus positive
75	0	0	26

**Table (2) Distribution of positive Rotavirus with gender groups
detection By Rapid test**

Gender groups	No. of positive	No of negative	total
Male	16	30	46
Female	10	19	29
Total	26	49	75

2-Reverse transcriptase PCR:

The reverse transcriptase PCR verified the results of the Rapid examination. the results of reverse transcriptase PCR for detection Rotavirus type A were indicated to 6 positive samples out of the 26 stool samples according to the findings in figure 1 and table 3 .

Figure 1: Rotavirus type A positive gel electrophoresis with unique RT-PCR product size (881 bp).

Table (3) Diagnosis of Rotavirus type A by RT-PCR

No . of stool samples	No. of Rotaviruses	No . of Rotavirus type A
75	26	6



Figure (1) Detection of Rotavirus type A by RT-PCR. The electrophoresis was performed at 100 Volts and 80 Amperes for 1 hour.

Lane (1 and 2, 4,6,) showing the positive results of human Rotavirus A at specific RT-PCR product size (881 bp).

Rotavirus type A is the most prevalent infectious agent responsible for acute viral gastroenteritis in children younger than three years old. These viruses are responsible for diarrhoea in young mammalian and avian species, including simian, porcine, bovine, ovine, caprine, equine, canine, feline, and murine [7]. Avian species include chicken, turkey, and pigeon.

The recent studies were used one-step chromatographic immunoassay demonstrate the presence of rotavirus antigen in stool samples. The principle of this method depend on reaction between antigen and antibody . many studies were used this method in diagnosis Rotavirus and other viruses because the chromatographic immunoassays are easy , low-cost equipment and basic experience, both of which are readily available in many laboratories while PCR technique has been used by some researchers to detect rotavirus infection[8]

The current research was used various approaches to detect the presence of rotavirus in stool samples of children with viral gastroenteritis called immunochromatography (ICA) and reverse transcriptase PCR in diagnosis of Rotavirus type A virus. The current research was done RT-PCR to confirm the identification of Rotavirus type A using the VP7 gene. Also, in the RT-PCR process that is used for, certain positive isolates are subjected to the RT-PCR

technique. Our study accepted with many studies [8,9] that used RT-PCR to determine rotavirus type A infection in children under the aged three years who had diarrhea.

RT-PCR was used to detection of rotavirus type A infection in stool samples from children suffering from acute diarrhea and it was compared to the Immunochromatography Assay (ICA). These findings suggest that RT-PCR is a sensitive and specific assay for detection rotaviruses type A in stool samples from people who have experienced acute diarrhea. This research accepted with others studies [8,9,10]that used RT-PCR to detection types of rotavirus in stool samples from patients with gastroenteritis. This research was similar to another study in a neighboring country such as Iran [11], which used RT-PCR to diagnose rotavirus genotyping in children's stool samples. A similar research in Nigeria used the same approach in diagnosis based on the RT-PCR technique's accuracy and sensitivity.

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