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Immunological Detection Of Rotavirus In Children With Acute Gastroenteritis In Al-Muthanna Province

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Abstract

Objectives: Rotavirus is the commonest agent answerable for intense gastroenteritis, in little young children . In Iraq, little information are recorded about this viral disease.

Methodology: This investigation meant to recognize rotavirus in fecal examples of 85 diarrhea cases, were gathered in Women and Children Teaching Hospital/Al-Muthanna Province/Iraq.

Results: Rotavirus antigen was detected in 25 (29.4 %) of the samples included the suspected cases and 24 (28.2%) excluded the suspected cases. The females were the most infected between the children and the age group of (10 ≥ months) recorded the highest infection rate. **Conclusions** : High prevalence of rotavirus among Iraqi children with acute diarrhea.

Keywords: Rotavirus – Reoviridae – Diarrhea – Gastroenteritis – ELISA .

Introduction:

A variety of causes, including parasites, bacteria, and viruses can cause extensive gastroenteritis with diarrheal illness; yet, intense gastroenteritis remains a critical general health crisis (Jaff, 2016) . According the (WHO), 2-million incidents of diarrhea are seen each year, and 1.9 million children under the age of five die due to acute gastroenteritis (Celik, 2015). Enteric viruses have been perceived as the most huge etiological agents of the disease, and four classes of viruses are being considered clinically pertinent: groupA rotavirus (family: Reoviridae), norovirus (family:Caliciviridae), adenovirus 40/41 (subgenus F), and astrovirus (Levidiotou, 2009). Rota virus is the commonest cause of viral diarrhea in newborns and children (Levinson, 2004), it is related for over 20% of diarrhea-related deaths in children under the age of five worldwide (Nguyen, 2004).

Children in poor nations die at a greater rate due to a variety of conditions, which included an absence of access to hydration medication and a greater percentage of starvation (Nakawesi, 2010). The WHO suggests that all national vaccination protocols include the rotavirus vaccine for infants (WHO, 2009). Several studies indicate contradictory results of the dietary habits impact on the risk of rotavirus diarrhea, while others found that diarrheal infection occured outside the residence, sanitary conditions of poor food, dehydration, educational levels, home stays with fewer than five rooms, containers, premature birth, maternal-smoking, male gender and maternal age under 20 years all increased chances of diarrheal rotavirus (Nakawesi, 2010). A- group of

Rotavirus is mostly possible cause of gastro-enteritis and may be diagnosed with several tests such as LA:latex-agglutination and EIA : enzyme immunoassay (Al-Sayidi, 2014).

Absence of inflammation in contaminated digestion tracts distinguishes viral-induced diarrhea from bacterial-induced diarrhea. Rotavirus contaminates intestinal villus enterocytes and crypt cells are preserved (Greenberg and Estes, 2009). Diarrhea by rotavirus can be associated with a number of conditions, notably (I) malabsorption, which can occur without the loss of enterocytes, (II) villus ischemia and enteric nervous system stimulation, which can be prompted by the release of a vasoactive agent from infected epithelial cells in the absence of considerable pathologic injuries or intestinal epithelium damage, and (III) intestinal release induced by the intracellular or extracellular activity of the rotavirus non-structural protein, NSP4, an innovative enterotoxin and secretory agonist with features of pleiotropy (Estes, 2001).

Materials and methods

Samples collection: .1

Stool specimens were gathered from (85) children suffering from gastroenteritis in Women and Children Teaching Hospital at Al-Muthanna Province/ Iraq. The specimens were collected at different times of a day, in to a fecal sample collection container with the use of a sanitized disposable spoon, about (1 - 2 ml or 1 - 2 g of solid sample) were gathered. The stool specimens finally stored at frozen condition (- 20 ^{o}C) until use.

2. Principle of the test

This test is devised, developed, and manufactured to assess the Ag of rotavirus in test specimens qualitatively. A microplates based enzyme immuno-assay is used in the test method that includes depositing higher purity antibody on the microtiter well's wall. Assay standards and feces, and even a Horseradish peroxidase conjugated monoclonal Ab that detects internal protein of rotavirus capsid, placed in microtiter wells of a micro-plate with a higher purity polyclonal anti rotavirus Abs on the wall. An immnocomplex of " Anti- rotavirus Antibody – Rotavirus antigen – HRP conjugated Anti-rotavirus Tracer Antibody" may formed after an incubation period if there was Rotavirus Ag in the sample. Unincorporated tracer Ab and another protein will be wiped away in the next step. After that, a spectrophotometric microplate analyzer is used to incubate the HRP-conjugated tracer Ab linked to the well. The proportion of rotavirus Ag in each test subject is roughly related to the tracer Ab enzyme activity which attached the rota virus immobilized on each microtiter well's wall (EDI^{TM} Fecal Rotavirus Antigen ELISA Kit).

3. Reagents :

Anti- Rotavirus Antibody coated microplate (96 wells) . -
Anti – Rotavirus Tracer Ab . -
TracerAb diluent . -
Wash concentrate (30 ml) . -
ELISA Horseradish peroxidase HRP substrate . -
ELISA stop_solution . -

Positive and negative controls . -
Concentrated patient sample diluent. -

4. Sample preparation:

Before being measured, the sample of patient had to be reduced 1:11 with sample diluent solution of patient. 100µl of liquid feces sample was put to this tube after labeling test tubes (1.5 ml plastic vials) and adding 1 ml of diluent to each vial. An equivalent amount of solid feces sample was obtained using a spatula, then the solid stool sample was suspended in 1ml diluent for patient sample and mixed well in a vortex. After allowing the diluted specimen to settle for around 5 minutes, the supernatant was employed in the experiment right away.

5. Assay Procedure

According to the manufacture instructions of (EDI™ Fecal Rotavirus Antigen ELISA Kit), The holder was filled with a suitable count Anti-rotavirus antibody-coated microwell. Microwells were filled with 100µl of controls and diluted stool samples, followed by diluted tracer Ab solution in every well of 50 µl. After an hour of incubation, the dish was rinsed five times. After 20 minutes of incubation, the HRP substrate was added, followed by the stop solution. The absorbance was measured using a microplate reader at 450 nm.

6. Results calculation :

Using the following formula, calculate the positive and negative cut-offs :

+ve (cut-off) = (mean absorbance of positive control + 0.08) *1.1

-ve (cut-off) = (mean absorbance of negative control + 0.06) * 0.9

Results and discussion

Table (1) shows the demographic characteristics of the (85) children infected with gastroenteritis divided according to age by months and gender.

Table (1): The Distribution of Demographic Characteristics

Item			Frequency	Percentage
Age groups / Months	I	10 ≥	43	50.5 %
	II	11-20	24	28.2 %
	III	21-30	17	20 %
	IV	31-40	1	1,17 %
	Total		85	100%
Gender	Male		35	41.1 %
	Female		50	58.8 %
	Total		85	100 %

The female seemed to have the greatest infection rate (58.8%) of diarrhea cases, whereas the male would have the lowest infection rate (41.1%), the age group of (10 ≥) months was the superior while the age group of (31-40) months were the least infected between the subjects

. In the case of acute gastroenteritis, the diagnosis of active rotavirus infection has been used to make a qualitative immunoenzymatic determination of rotavirus antigen. The cut-off rate of developing IELISA was determined using formula mentioned in used kit, it was the OD value of less than (0.96) which mean those results are negative, higher than (1.18) will be considered as a positive, and between (0.96 – 1.18) will be considered a suspected. The results of ELISA assay excluded the suspected cases ($ELISA_{ex}$) were 24 (28.2%) positive cases and 61 (71.7%) negative cases from 85 gastroenteritis patients. The positive cases, according to age groups(I, II, III and IV) as follows (18.8% , 5.8%, 3.5% and 0%) respectively as shown in Table (2).

Table (2) : Rotavirus infection percentage excluded suspected cases.

Item			Infection Percentage	p. value
Age groups / Months	I	≥10	18.8 %	0.3
	II	11-20	5.8 %	
	III	21-30	3.5 %	
	IV	31-40	0 %	
Gender		Male	11.7 %	0.9
		Female	16.4 %	

*** significant at p ≤ 0.05**

As it seems there was no significant differences in infection between the four age groups, this indicate that Rotavirus may infect all ages in children under 40 months. But the age group of (10 ≥) months was the most infected group, this may particularly compatible to Nakawesi et al. who found most of children (95%) with diarrheal rotavirus were less than twenty-four months (Nakawesi et al., 2010).

The most significant patient aspect that impacts the infection's clinical prognosis is age. As a result, infants infected with rotavirus experience symptoms rarely; this protection is considered to be mediated primarily by maternal antibodies transmitted across the placenta (Ray et al., 2007). Reduced antibody levels correspond to the age when newborns are most vulnerable to rotavirus infection (3 months to 2 years). Adults can be infected with rotavirus, however serious clinical disease is unusual. Infections with an atypical virus strain or extremely large viral doses can induce severe clinical illness (Greenberg and Estes, 2009).

Our results of an indirect ELISA included the suspected cases ($ELISA_{in}$) were 25 (29.4%) positive cases and 60 (70.5 %) negative. The positive cases, based on age groups were as follows (20%, 5.8%,3.5% and 0%) respectively as shown in Figure (1). The suspected case was female with age of 4 months, its OD value was between the positive and negative cut-off.

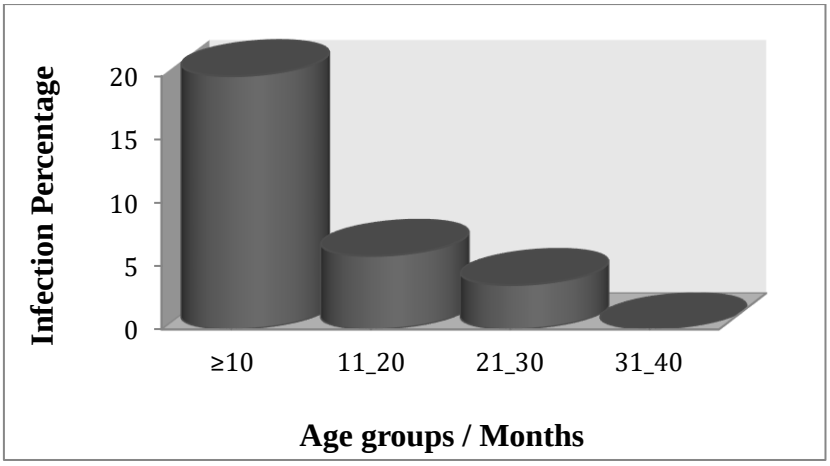

Fig (1) : Percentage of infection between age groups included suspected cases.

Table (2) illustrates that there was no substantial difference (p. value = 0.9) between males and females infected with Rotavirus using the X^2 test, but females seem to be the most infected (fig 2), which contradicts a study in Baghdad, observed that male were more vulnerable than female to viral infection and that the differences were substantial between them (P< 0.05)(Al-Sayidi et al., 2014). In another research, males had a greater rate of viral infection (13% vs. 9% for females) (Jaff et al., 2016).

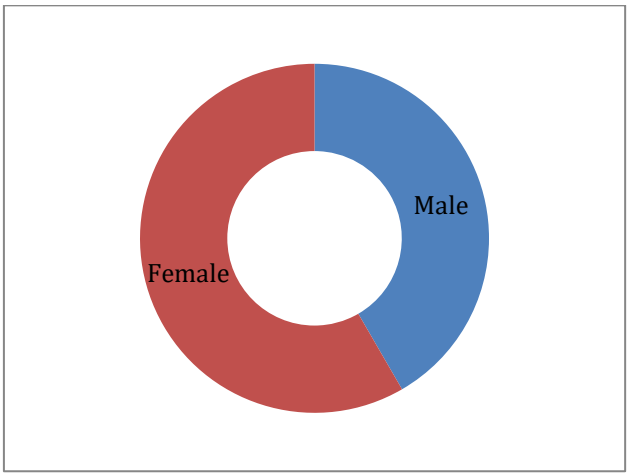

Fig (2): The proportion of patients infected with rotavirus varies depending on their gender

Conclusions : High prevalence of rotavirus among Iraqi children with acute diarrhea. Early recognition will contribute in the successful handling of the cases.

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