

Full Length Research Paper

Detection of Rotavirus in diarrhea stool samples of children with acute gastroenteritis in Babylon governorate, Iraq

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Abstract

Human rotaviruses are the most important etiologic agents of acquired diarrhea in infants and young children worldwide. Early diagnosis is essential for effective patient treatment. Three different commercial diagnostic kits, latex agglutination (LA), test device and ELISA were evaluated for rotavirus detection in diarrheic fecal samples of children with acute gastroenteritis. Two hundred thirty six diarrheic fecal samples were collected from beginning of October 2011 to end of March 2012. Specimens were analyzed for detection of Rotavirus, (108) samples were positive by Latex and ELISA test, whereas (112) are positive by test device method. The sensitivity and specificity of LA were (97.2%), (97.1%) respectively, while the sensitivity and specificity of stripe were (97.2%), (94.5%) respectively. These results indicate that latex assay is sensitive and specific as the ELISA, and it could be applied on a large scale for screening stool specimens in suspected rotavirus diarrhea. Whereas test device has similar specificity and lower sensitivity in diagnosis of Rota virus. However, the indeterminate results must be confirmed by other methods, such as RT-PCR.

Keywords: Rotavirus gastroenteritis, Latex, ELISA, test device (stripe).

INTRODUCTION

Diarrhea diseases in children are a major public health concern in developing countries, including Iraq and neighboring countries. Viruses cause about 70% of episodes of infectious diarrhea in the pediatric age group (Webb and Starr, 2005). Rotavirus is the most common cause of gastroenteritis in children, and produces similar incidence rates in both the developed and developing world (Meloni et al., 2011; Szajewska and Dziechciarz, 2010). In the 1969, Rotavirus was discovered in Nebraska calves, and in 1973 was first described in humans, when electron microscopy images reveal its presence in duodenal biopsies of children with acute gastroenteritis (Bishop et al., 1973). Rotavirus is a less common cause in adults due to acquired immunity (Desselberger and Huppertz, 2011).

Diarrhea is a major cause of malnutrition in children, predisposing them to other infectious diseases that increase child mortality in these countries. Estimates of mortality in developing countries reveal 4.9 children per 1,000 per year die as a result of diarrheal illnesses in the

first five years of life. This contributes to the childhood mortality in children younger than five years by 21%, with most deaths occurring during the first year of life (Bern et al., 1992; Bresee et al., 1999). Acute gastroenteritis is among the most common illnesses of humankind and its associated morbidity and mortality are greatest among children and the elderly (Wilhelmi et al., 2003). Rotaviruses are the most important cause of severe dehydrating diarrhea in young children in both developed and developing countries (Alrifai et al., 2009). Nearly all children are infected by a rotavirus at least once by the age five years (Forster and Hammerschmidt, 2007). Rotavirus is the most common cause of severe infantile diarrhea worldwide, 130 million children are estimated to develop diarrhea due to rotavirus each year, resulting in more than 611,000 deaths (Institute of Medicine, 1986).

Since rotavirus disease cannot be eliminated through improvements in water and sanitation, development of a safe and effective rotavirus vaccine has been a priority (Fischer et al., 2007). The prevalence of rotavirus infec-

tion, serotype characterization, and epidemiological distribution of Rota viral infections have been extensively studied in developed countries where rotavirus vaccination has been implemented. In developing countries where rotavirus infection, morbidity and mortality are high.

In Iraq, the death rate in children <5 years of age was reported to be 130/1,000 for boys and 120/1,000 for girls in 2003 (World Health Organization, 2003).

Diarrhea is a major cause of illness and death in Iraqi children. However, several Iraqi researchers recorded different percent of infectivity with Rota viruses among Iraqi governorate. Rotavirus accounted for 24% of acute diarrhea in hospitalized children in Basra, 37 % in Arbil, 18.5% in Tikrit and 30% in Baghdad (Abdulrazzaq et al., 2011; Alrifai et al., 2009; Herish et al., 2006; Mahmood and Feachem, 1987). Therefore this study was carried to estimate the prevalence of rotavirus in Babylon governorate using three commercial diagnostic kits and will be followed with study for detection of rotavirus genotyping circulating between populations using RT-PCR molecular diagnostic kit.

MATERIALS AND METHODS

Sample collection

The study was conducted from October 2011 to March 2012. Two hundred thirty six diarrheic fecal samples from outpatients and hospitalization children with acute gastroenteritis in Babylon governorate were tested by (LA), test device and ELISA for rotavirus antigen detection. The specimens were collected in sterile containers, and immediately processed with PBS buffer solution, (pH 7, 2), 10% suspension of each sample was prepared sterile clean plain tubes for performing latex test and ELISA test. Samples were homogenized thoroughly in a vortex mixer, whereas the remaining amount of stool samples were stored at-40°C till used in other assay procedures. The homogenized suspension was clarified by centrifugation at 2,000 rpm for 10 minutes. The supernatant solution was separated, the LA, ELISA and stripe tests was performed.

Latex test for human Rotavirus Antigen

LA was performed by using the commercial latex agglutination kit (**Blasmatec, Germany**). The test was considered positive for rotavirus if distinct agglutination was observed with test latex but not with control latex and indeterminate if agglutination was observed in test and control latex, this test was performed according to the manufacturer's specifications.

ELISA test for human Rotavirus Antigen

The Rotavirus Antigen Detection ELISA kit (**DIAGNOSTIC AUTOMATION, INC.USA**) is a qualitative determination of rotavirus antigen in feces. This test was performed according to the manufacturer's specifications.

Detection of rotavirus by (One step rotavirus test device)

The one step rotavirus test device (**Acon, Germany**) is a rapid chromatographic immunoassay for the qualitative detection of rotavirus in human feces specimens to aid in the diagnosis of rotavirus infection. This test was performed according to the manufacturer's specifications.

RESULTS

Positive rotavirus antigen was detected in 108 out of 236 samples (45.76 %) by LA and ELISA, and (112 out of 236) samples by test device (Table 1).

Results of rotavirus detection by LA test reflecting a sensitivity and specificity of 97.2%, 97.1%, respectively, whereas sensitivity and specificity of test device were (97.2%), (94.5%) respectively.

The comparisons were made on the assumption that the specimens tested with ELISA were genuinely positive or negative. Of the total rotavirus positive patients, 55.14% were male and 33% were female (Figure 1).

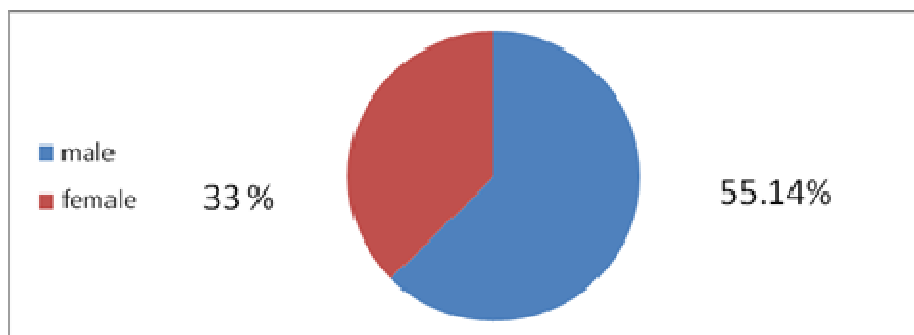
The majority of rotavirus-positive cases were observed in children younger than 5 years and children age group of 18-24 month old reflecting the highest percent of infectivity (61.5%) where as age group of 24 – 60 month old revealing the lower percent of rotavirus infectivity (32 85%) Table 2.

DISCUSSION

Rotavirus was detected in 108 out of 236 stool samples of diarrheic patients studied by latex, ELISA whereas 112 out of 236 were detected by test device (stripe). The majority of the cases occurred in children younger than 2 years, which is the susceptible expected target age group. In slightly older children, rotavirus infection can be asymptomatic; probably because they have some degree of protection from clinical disease owing to previous infection with this agent (Bos et al., 1995). Rotavirus infection has been observed to follow a seasonal pattern in our region, where it is associated with cooler temperatures and a drier atmosphere. The seasonal distribution of this infection during the cooler months has been reported before for temperate regions (Bos et al., 1995).

Table 1. Comparison of the Results Obtained by Enzyme linked Immuno sorbent assay, Test device and Latex Agglutination tests.

TEST	Specimens Number	Positive	Percentage	Negative	Percentage
Latex test	236	108	45.76	128	54.23
ELISA test	236	108	45.76	128	54.23
Test Device	236	112	47.46	124	52.54

**Figure 1.** Distribution of rotavirus according to gender.**Table 2.** distribution of rotavirus according to age.

Age (Months)	No. of specimens	Positive		Negative	
		No.	%	No.	%
0 – 5	5	2	40	3	60
6 – 11	45	23	51.1	22	48.8
12 – 17	85	43	50.5	42	49.4
18 – 23	13	8	61.5	5	38.4
24 – 60	70	23	32.85	47	67.1
>60	18	9	50	9	50
Total N.	236	108	45.76	128	54.23

The rapid diagnosis of rotavirus infection in patients admitted to the hospital with symptoms of gastroenteritis would enable better treatment of the patient, such as isolation or discharge, as in many cases effective rehydration can be achieved at home, and most rotavirus infections are self-limiting (Bos et al., 1995). An accurate diagnosis of rotavirus is essential since it obviates the unnecessary use of antibiotic therapy. The methods of choice for the detection of rotavirus in stool samples should have high degrees of sensitivity and specificity, high predictive values, and reproducibility, which ensure consistency of performance in the laboratory.

ELISA test kits for detection of viral antigens is the method commonly used in many laboratories in combination with either electropherotype determination by PAGE or detection of viral particles by electron

microscopy, as well as other conventional methods used for detection of viruses (Agius et al., 1984; Argüelles et al., 2000).

Each of these assays, however, inherently possesses its own characteristic limitation (Jensen A (1985). Evaluation of two commercial kits for rapid detection of human rotavirus in feces: Rotalex, a latex agglutination test and rotavirus ELISA kit. Acta. Pathol. Microbiol. Immunol. Scand B. 93(2):159-60.

Lipson and Zelinsky-Papez, 1989). For rotavirus infection, in which levels of virus shedding are usually very high, all of these methods are suitable for diagnostic purposes. Considering that culture methods for human rotavirus stool samples are reported to be 75% as efficient as antigen-detection methods, culture procedures are not considered the "gold standard"

against which other tests may be judged (Glass et al., 2006). The LA test has been reported to be a simple, sensitive, rapid test and is recommended for field studies in which speed and simplicity are crucial (Jensen, 1985). Many reports have been related comparing LA with other tests, and variable sensitivity and specificity of the LA commercial kits were observed. Some authors reported that only a modified procedure gave results comparable with those obtained by electron microscopy, immunofluorescence, PAGE, and ELISA (Moosai et al., 1985).

Raj *et al.*, (1889) reported that the LA kit studied was a specific and rapid method. While it may be useful in certain situations, such as in outbreaks, its low sensitivity can make it unsuitable for use in routine clinical practice (Raj et al., 1989).

Since 1989 till now several development were introduced by manufacturing companies for latex test so its sensitivity and specificity can increase if producer use monoclonal antibodies. The present study demonstrated that the degree of sensitivity and specificity of LA test showed a roughly linear relationship with the degree of ELISA sensitivity and specificity, and the positive predictive value of the test was high. Thus, the simple and inexpensive LA test would be useful as a screening procedure to detect rotaviruses in stools of children with diarrhea. Therefore, it is possible to conclude that strongly positive reactions with the LA test may be regarded as true-positive reactions. However, samples producing weakly positive or indeterminate reactions should be retested in a more specific and sensitive assay, such as ELISA (Amer et al., 1990; Grauballe and Jarzabek, 1984).

In present study we concluded that LA kit is of low complexity, is easy to interpret, and provides a rapid diagnosis in a short time. It reflected a high degree of sensitivity and specificity. It is a suitable screening test for the diagnosis of rotavirus gastroenteritis and better than test device stripe. The test device were applied first time in this study for comparison with LA as a commercial available kit in market as cheaper diagnostic kit, yet results revealed that its sensitivity similar to LA and ELISA(97.2%), but its specificity less than the other two test(94.5%). Variable results were observed with different LA kits, so it is recommended that before this test is used routinely, different commercial kits must be compared in separate study to decide which one is better depending on result, and as it is much cheaper than ELISA and faster in time performance; therefore, we advise to use it in routine laboratory work, whereas diarrheic stool sample of doubtful result or sure to be of rotavirus cause can screen by other confirmatory assay like ELISA or RT-PCR molecular technique.

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