Performance of Latex agglutination, ELISA and RT-PCR for diagnosis of Rotavirus infection

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Abstract

The rotavirus is one of the major factors of inducing the acute gastroenteritis infection in children under 5 years of age. The laboratory diagnosis is progress and bringing it under control as well as avoiding its diffusion. The purpose of the present study was to determine the performance of enzyme linked immunosorbent assay (ELISA) and Latex agglutination (LA) tests against reverse transcription-polymerase chain reaction (RT-PCR) for evaluating the children's acute gastroenteritis by rotavirus.

One hundred feces specimens were collected from February to May 2014 and analyzed by LA, ELISA and RT-PCR.

In this study, the positive results for rotavirus detected by ELISA, LA and RT-PCR were 37, 43 and 27%, respectively. In addition, the result showed that the sensitivity and specificity of ELISA and LA were 74 and 85%, respectively, when compared to RT-PCR.

For laboratory detection of Rotavirus infection, RT-PCR has the highest sensitivity and specificity but because of the high costs, ELISA and LA based kits with good performance, as shown by this study, can be preferred for the routine use.

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Introduction

According to the World Health Organization, nearly 20 to 30% of acute diarrhea cases known around the world are ultimately turned into lasting diarrhea, leading to poorly digested food, malnutrition and developmental insufficiency in the children.[1] Amongst the factors inducing gastroenteritis, the viruses are the most common ones and the Rotavirus, Astrovirus and Adenovirus are most significant.[2] Thirty three percent of diarrhea cases are caused by rotavirus.[3] It is one of the causative agents of acute gastroenteritis in infants and young children around the world which is transmitted by fecal-oral root[4] and also, belongs to the Reoviridae family and has a 20-fecet symmetry (Icosahedra), lack of coverage, and its genome is a double stranded RNA (dsRNA) and is formed by 11 segments.[5,6] According to the reports, around 527000 children under 5 years old are dying annually as a result of rotavirus gastroenteritis. Over 85% of mortality occur in the low-income Asian and sub-Saharan African countries due to rotavirus.[7] Every child under 5 years old is infected with rotavirus gastroenteritis at least once.[8]

It is seen that the children under 2 years old are mostly affected by rotavirus infection. This infection is a self-limited one; however, as a result of highly extracted body fluids as well as imbalance of electrolytes, the affected ones may be facing death, particularly in the developing countries.[9] The function of conveying the nutrients to villi's cells of small bowel is disrupted by rotavirus without affecting the gastric mucosa and colon.[10] The ratios estimated for the infection in Iran vary in different parts, ranging from 11.36% in Shiraz to 67.64% in Mazandaran to 79% in Tehran. The various variables such as geographical locations, periods of time, seasons and way of virus detection, age groups, gender and controlling the presence of non-diarrhea pathogens are involved in examination of disease prevalence.[8]

The viral particles are comprised of 3 protein layers. The structural proteins of VP1-4, VP6, and VP7 associated with non-structural proteins of NSP1-6 are encoded by viral genome.[11,12] The inner capsid is consisted of VP6 protein that is the most abundant viral protein. It is an appropriate target for detecting the infection caused by rotavirus.[13] The rotaviruses are assigned to seven groups of A to G based on VP6 protein. The rotavirus belonging to group A is the most involved virus in infecting the human. In addition, 23 types of G and 32 types of P are distinguished by VP4 and VP7 antigens. The initial signs of infection induced by rotavirus are acute watery diar-

rhea and vomiting and the patient needs to be hospitalized because of water deficit of the body.[8]

Several techniques for rotavirus diagnosis have been developed. The detection of rotavirus factor was first conducted by electron microscope, followed by techniques such as polyacrylamide gel electrophoresis (PAGE), immune-fluorescence (IF), radioimmune-assay (RIA), reverse passive hemagglutination (RPH), enzyme-linked immunosorbent (ELISA) Latex agglutination test (LA) and, more recently reverse transcription-polymerase chain reaction (RT-PCR) and immunochromatography (IMC).[13]

The aims of this study were to compare the function of three laboratory techniques to detect the rotavirus infection in children younger than 5 years of age with acute gastroenteritis.

Materials and Methods

Sample collection

This study was conducted in Aboozar children's hospital, Ahvaz city from Iran, hence, all the feces samples were collected on February to May 2014. The feces samples were taken from one hundred children who referred to the hospital with clinical signs of diarrhea, vomiting and fever. Using a questionnaire, the personality traits and nutritional habit of each patient along with the clinical symptoms such as diarrhea, vomiting, fever, blood in the stool, convulsions, runny nose, past history and disease severity were considered and recorded. Meeting such requirements as having an age less than 5 years and signs as diarrhea, vomiting and fever for less than 2 weeks was required for sampling, also, the clinical symptoms depending on the pathogen and the host defense mechanism is variable.[14]

Before sending the samples to the Research Center for Virologic Examination, the possible pathogens of bacterial or parasitic origins were studied together with the WBC and RBC counts in the blood. Each sample negatively detected in the Lab was poured into three different microtubes and by observing the cold chain and placing the samples on the ice, they were transferred to the laboratory of virology department in Ahvaz Jundishapur University of Medical Sciences and had been stored at –70°C for varying times before use. In order to test the ELISA, the Generic Assay Germany Kit with a sensitivity of 98.4% and specificity of 100% was used and to do the Latex the Omega Diagnostic, England Kit with a sensitivity of 97.2 and specificity of 97.1% was introduced and registered in the Kit manual.

Latex agglutination

This test in term of qualitative detection is very rapid for diagnosing the rotavirus antibody. Nevertheless, the sensitivity and specificity of LA may vary according to the commercial kit used. The feces specimens were tested by LA Test [(Virotect ROTA OD038), Omega, England], the particles of latex were coated with antibody of antivirus. The sample was considered positive for rotavirus when agglutination was observed within two minutes reaction, as recommended by the kit manufacturer.

Enzyme-linked immunosorbent assay

ELISA is a simple technique that enjoys some advantages as portability of the equipment, hand-holding validation, and reliability for the assessment of samples.[15-17] All feces samples were tested by commercial ELISA test kit (Generic Assays, Germany) according to the method described by manufacturer's instructions.

Reverse transcription-polymerase chain reaction

The extraction of viral RNA was conducted by utilizing the RNA purification and Gene JET Viral DNA Kits, made by Ferments Co, South Korea. It is notable that the extracted RNA was stored in the freezer –70°C until fabrication of cDNA. In this phase, the extracted RNA was transformed to CDNA using AccuPower® CycleScript RT PreMix (dN12) Bioneer Co, South Korea.

Values for PCR reaction were as follows:
VP6-Forward: GACGGV(c)GCR(b)ACTACATGGT[18]
VP6-Reverse: GTCCAATTCATN(d)CCTGGTG [c=(N=A,T,C or G), b=(R=A or G),d=(Y=C or T)].[19]

The reaction mixture contained 2.5 µL PCR reaction buffer 10X (Cinnagen, Iran) with 0.25 $MgCl_2$ 50 mM (Cinnagen, Iran), 0.5 µL dNTPs 10 mM (Cinnagen, Iran), 0.2 µL of Taq DNA polymerase 2U (Cinnagen, Iran) , 0.25 µL of each primer (100 mol) and 2 µL of the template. PCR was performed on Techne Thermal Cycler (UK) for 35 cycles. Cycling conditions were as follows: 95°C for 10 min; 35 cycles at 94°C for 45 sec, 55°C for 45 sec, 72°C for 45 sec, and a final elongation at 72°C for 10 min. The expected PCR product was 382 bp. The PCR product was subjected to electrophoresis on a 2% agarose gel, stained with DNA safe stain, and observed under ultraviolet light. For the positive control, positive samples in the samples archive which had previously been sequenced were used[20] and for the negative control, the sterile deionized was water.

Statistical analysis

The data was analyzed by SPSS for windows TM version 19 and Microsoft Excel for windows 2007 using Pearson test (rho) for correlation between the variables. The differences among the mean values were found to be significant at $P \leq 0.05$.

Results

In this study, 100 children with acute gastroenteritis by the age range of 1 to 60 months to detect rotavirus antigens in stool samples were studied. There rotavirus in 27 samples were found positive by RT-PCR test of which only 26 cases were positive immunoferment 24 by LA test. ELISA a sample of 62 negative samples (1.61%) and 54 negative samples latex three samples (5.55%) were detected positive by RT-PCR test. By comparison, the sensitivity and specificity for LA test and ELISA were 89, 74, 96 and 85%, respectively. Comparison was made on the assumption that the samples tested by RT-PCR were positive or negative. Also, the PPV, NPV, FP and FN have been reported in Table 1.

Among the positive samples with rotavirus, 59.25% were male and 40.74% were female. The majority of positive cases of rotavirus in children under 2 years were 88/89% and the largest number was among the 6 to 8 month age group (29.62%) was observed in Table 2.

Discussion

Rotavirus (RV) is the main etiological agent of diarrhea in infants and young children worldwide, accounting for 30 to 50% of acute diarrheal illnesses; hence, its laboratory diagnosis is crucial to guide the clinical management and prevention of its spread.[20,21]

The specific detection of produced infection by rotavirus is carried out by studying the feces samples through Enzyme Immunoassay techniques with diagnostic Kits, for its having high

detection speed, high sensitivity and specificity; and are applied for diagnosing all the serotypes of rotavirus.[22,23] In addition, these kits are employed for detecting the animal rotavirus infection.[22] The most used tests in detecting the rotavirus infection are electron microscope, LA, ELISA, Immunofluorescence PAGE as well as molecular tests.[24] In this study, the comparison of ELISA and LA methods compared to RT-PCR and the result showed higher sensitivity and high specificity of RT-PCR. The highest sensitivity was (96%) obtained for ELISA followed by LA (89%). While the highest specificity was (85%) obtained for ELISA followed by LA (74%). Moreover, these results calculated for the positive predictive value (PPV) of ELISA and LA were 70.3 and 55.81%, respectively, and, the negative predictive value (NPV) of ELISA and LA were 98.4 and 94.74%, respectively. Although the rapid detection of viral specimens is easier with ELISA and LA methods, the disadvantages of these methods in this test are high for FP and NP values. Each factor is due to the high values of these parameters, including how samples are collected, how samples are transferred, or how any action that contributes to changes in the number of rotavirus in the samples are taken.

The previous survey by Buesa *et al.*[25] compared RT-PCR, ELISA, PAGE and electron microscope. The positive rate was obtained as 30, 29, 26.85 and 25.45% for PCR, ELISA, PAGE and electron microscope, respectively. Although, the results have shown that LA for detecting the presence of antigen is associated with high sensitivity and good specificity, but ELISA test is of good sensitivity and high specificity. Both tests easily detect the infection with high speed. Nevertheless, the LA test is used in detecting the rotavirus in laboratory of hospitals or in private practice of the physicians for fast diagnosis.[26] However, the ELISA can be utilized for screening the aggregate.[27] Also, the other study by Steele *et al.*[28] revealed that two methods by ELISA and LA were compared. They argued that the ELISA is fast and with significant sensitivity (100, 96%) and can be used for aggregate screening that is consistent with our results. The conducted research study by Eing *et al.*[22] examined two different ELISA, labeled as RIDASCREEN® rotavirus and Path finder rotavirus, in 393 patients. Both tests had 100% sensitivity as well as positive predicative value 93.7 and 57.7%, respectively. The previous survey by Ibrahim *et al.*[29] reported that LA has a high sensitivity of 96% but its specificity is the lowest. Also, the electron microscope and PAGE have 100% sensitivity and their specificity is 73 and 84%, respectively.

The other survey from Baghdad, which examined the performance of ELISA and LA in detecting gastroenteritis infection, showed the sensitivity and specificity for ELISA and LA were 92.5, 86.3% and 84.09, 93.6%, respectively. The reaction percentage of false positive for LA was 7.7% over that of 3.3% for ELISA.[13]

In a survey by Altindis *et al.*[24] it was reported that the three methods of LA, ELISA and gel electrophoresis polyacrylamide were used for detecting rotavirus. Being positive for rotavirus test was reported by three above methods as 12.59, 15.55 and 11.85%, respectively. The sensitivity and specificity of ELISA and LA over PAGE were expressed as 100, 99.16 and 93.75, 94.96%, respectively. Both methods had high sensitivity and specificity. The other study showed that the laboratory detection of rotavirus infection and the effect of immunizing on hospitalization. By comparing the three methods of ELISA, LLA in detecting rotavirus antigen and PAGE in detecting RNA, they concluded that the ELISA was more specific with higher sensitivity of 94.6% and specificity of 94.4%, accuracy of 94.5% and high detection speed. The percentage of being positive for ELISA, LA and PAGE was obtained 28.3, 34.8 and 25.6%, respectively. Also, this study showed the occurrence of rotavirus infection since implementation of global safety programs. For LA test, the sensitivity was obtained 82.6 and 81.6% which are consistent with our results.[30]

Table .1 Compare ELISA and LA test against RT-PCR test.

Parameters	LA%	ELISA%
Sensitivity	89	96
Specificity	74	85
Positive Predictive Value (PPV)	55/81	70/3
Negative Predictive Value (NPV)	94/74	98/41
False Positive (FP)	44/2	29/7
False Negative (FN)	5/55	1/61

Table 2. The positive characteristics of the study sample.

Parameters	Rotavirus positive samples (N=27)	
	n	%
Sex	16	59.25
Male		
Female	11	40.74
Age (month)		
0-12	16	59.25
13-24	8	29.63
25-36	2	7.41
37-60	1	3.7
Clinical symptoms		
Diarrhea	25	92.59
Vomiting	24	88.9
Fever	21	77.8

Conclusions

Rotavirus detection is greatest when diarrhea, vomiting, and fever occur together and lowest when each symptom occurred alone. Diagnosis of the infection is based on the identification of the virus in feces or suspension of rectal swab collected early in the illness through direct microscopy, molecular techniques, rapid serological tests, and the use of tissue culture technique. The results indicateed that the LA and ELISA kit used for rotavirus diagnosis presented good sensitivity, high specificity, and easy proceeding, providing fast diagnosis for rotavirus infections. Also, these techniques indeed influence clinicians in the implementation of effective management and control measures to pediatric rotavirus diarrhea disease. Hence, these methods may thus be used as a reliable test for diagnosis of rotavirus infection.

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