Research Paper

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Comparison of Immunochromatography with RT-PCR for Detection of Rotavirus in Fecal Samples

Medical Science

KEYWORDS : Rotavirus, Antigen detection, Diarrhea, Rapid ICT, RT-PCR.

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ABSTRACT

The study was conducted to detect the prevalence of rotavirus associated diarrhea in children less than 2 years of age and to evaluate the efficiency of rapid immunochromatographic test [ICT] in rotavirus antigen detection and also to compare its performance against Polymerase chain reaction [RT-PCR], the gold standard test. A total of 100 stool samples were collected from hospitalized children less than 2 years of age with loose stool without blood and mucus. All 100 samples were tested for rotavirus by both rapid immunochromatography and RT-PCR.Out of 100 children tested by rapid ICT, 34% were positive for Rotavirus antigen in the stool sample and 64% by RT-PCR .All 34 samples positive by ICT were positive by RT-PCR also. Of the both ICT and RT-PCR samples (34), 28(82%) samples had Ct values of less than 30 indicating a high viral load. Only 6(18%) samples had Ct value of more than 30 where as in samples which were negative by ICT and positive by RT-PCR (30), 29(97%) samples had Ct values more than 30 which indicates a lesser viral load. Rapid ICT had a sensitivity of 53.1%, Specificity of 100% when evaluated against RT-PCR as gold standard. Rotavirus is the major cause of acute diarrhoea in children less than two years of age. Highly sensitive detection method like RT-PCR is necessary to find out the prevalence of rotavirus infection, though ICT can be used as an alternate test for rapid diagnosis in emergency testing.

Introduction

Diarrhoeal diseases have been recognized in humans since antiquity. Diarrheal diseases are one among the most common illnesses in infants and young children all over the world. They are one among the six major causes of childhood mortality accounting for 18% of the deaths [1] in children less than five years, with highest mortality in developing countries [2]. Rotaviruses are the single most important etiological agent causing severe diarrheal disease in children less than five years of age in both industrialized and resource poor nations, responsible for 30-50% of these diseases.[3]

Almost every child will have an episode of rotavirus gastroenteritis by the age of five . Around 98,621 rotavirus-induced diarrheal deaths have been recorded India in 2008, which is about 22% of global deaths.[4]Rotavirus infections are responsible for 34% of all hospitalizations due to diarrhea [5] .The proportion of severe diarrhea attributable to rotavirus has increased from an average of 25% in studies which were completed prior to 2000 to over 38% in studies that were completed after 2005.

It is clinically not possible to differentiate the diarrhea caused by rotavirus and other infections. Therefore, an accurate diagnostic assay is necessary to establish rotavirus as a causative agent of gastroenteritis because children with rotavirus infections need not be given any antibiotic except for other co existing bacterial infections. Inappropriate use of antibiotics enhances the emergence of resistance in bacteria and also adds the cost of treatment and risks of adverse reactions. Several techniques have been developed to detect rotavirus in stool. At present rotaviral disease is confirmed viral antigen detection in stool sample. Rapid tests like immunochromatographic tests, latex agglutination and enzyme immunoassays are commercially available now. Rapid tests can only be used for screening but not for confirmation of infection..

Reverse transcriptase polymerase chain reaction (RT-PCR) is the most sensitive molecular method available to detect and confirm rotavirus when done with the appropriate choice of primers. [6].

Rotavirus infections are frequently underestimated since routine laboratory diagnosis is not done. Adequate surveillance and introduction of rotavirus vaccine into the Universal immunization programme are the important part of efforts to reduce diarrhea mortality which is the third leading cause of death among Indian children [7].

Materials and Methods

The present study was conducted at the Department of Microbiology, Tirunelveli Medical College, Tirunelveli from May to November 2012. A total of 100 stool samples were collected from eligible children of less than 2 years of age with loose stool, without blood and mucus. Ethical clearance was obtained from the college ethical committee before the commencement of the study. Informed consent was obtained from reliable informants of all patients. Symptoms regarding the onset of diarrhea, duration, presence of other clinical features like vomiting, fever and features suggestive of dehydration and history of rotavirus vaccination were recorded in a questionnaire.

Around 5 ml of sample collected in a sterile container with a spoon attached to the lid from the suspected cases in the acute phase of illness, preferably within 48 hours of hospital admission.

Samples were immediately tested for rotavirus by rapid immunochromatographic method. The presence of colored band ("T" band and "C" band) within the result window. Then the samples were stored in a buffer for RT-PCR at -80° C.

All the 100 samples tested by rapid ICT were tested again with RT-PCR by the kit provided by Helini Biomolecules, Chennai, India and the procedure was done according to the manufacturer's guidelines using primer specific for NSP3 region of rotavirus. In this study, real time Q-PCR was used detect rotavirus in stool samples. Amplification reactions were performed on a Biorad (CFX 96-Realtime system) Thermo cycler with specific Amplification profile. Results along with amplification curves are stored in the computer. Ct value <37 with typical sigmoid shape amplification curve was taken as positive .Ct value ≥37 are taken as negative.

Statistical Analysis

All the results obtained from the study were analysed stasticially by IBM SPSS Statistics 20.

Results

Out of 64 samples tested by RT-PCR 39 (61%) were in the age group of 7-12 months. Association of this age group with rotavirus positivity was statistically significant**. (Table-1)** Out of 64 children positive by rapid ICT 34 (53%) were male and 30 (47%) were female. There was no difference in gender related to rotavirus infection.

All the 100 samples tested by both rapid ICT and RT-PCR for rotavirus in their stool samples. Out of this RT-PCR was positive for 64% of samples while 34% were positive by ICT.

(Table-2&3) The rapid immunochromatographic card test was evaluated for its sensitivity and specificity against RT-PCR, a gold standard test. Sensitivity of rapid ICT was 53.1% when evaluated against RT-PCR, a gold standard test. Specificity was 100 % compared to RT-PCR and positive predictive value of ICT

was also 100%.Negative predictive value of ICT was 54.5%

The association of breast feeding with rotavirus positive cases was analysed in the above table. In 64 PCR positive children, 27(42%) were breast fed while 37(58%) were not. Among 36 PCR negative children, 23(64%) were breast fed and 13(36%) were not breastfed. It was found that breast feeding had a significant correlation with rotavirus positive and negative children. **(Table-4)**

Discussion

Rotaviruses are the most common cause of acute diarrheal diaease in young children leading to dehydration. Mortality is pronounced in developing countries like India. Diagnosis of this infection in children with diarrhea will help to estimate the real burden of gastroenteritis attributable to rotavirus. Identification of rotavirus by a method which is both sensitive and cost effective will improve the laboratory diagnosis. In this background this study attempts to evaluate the rapid immunochromatographic card test against RT-PCR in diagnosing rotavirus in stool samples of children less than two years. Stool samples were collected from100 hospitalized Children of less than 2 years of age with diarrhea over a period of one year.

Prevalence of rotaviral diarrhea was more common in the age group of 7-12 (61%) in this study .Similar finding was seen in a study done by M Kargar et al in 2012[8] and Vivek Jain et al [9] in India 2001. The incidence in 7-12 months was followed by the age group of 0-6 months. This may be due to breast feeding, exclusive or partial in the first 6 months and also due to the acquired immunity from the mother which may prevent infection in the first few months of life.

Present study revealed no association of sex with incidence of rotavirus infection .This is in contrast to the study by Kelkar SD,1999[10] and Trung Vu Nguyen et al [11] in Vietnam where male predominance was noted

The positivity rate of rapid ICT was 34% while it was 64% by RT-PCR. In the present study Rota virus accounts for 64% of hospitalization in children with acute diarrhoeal illness .The prevalence is comparable to a study conducted by Hamsa T Tayeb *et al* [12] in 2011.It is postulated that improvements in sanitation and use of antimicrobials have had a greater impact on preventing bacterial and parasitic gastroenteritis (GE) than rotavirus.

The sensitivity of ICT was low (53.1%) while the specificity was 100% when evaluated against RT-PCR. Similar low sensitivity was reported by Petra F. G. In the study by Wolffs et al [13] .Hence all the samples negative by ICT have to be confirmed by a more sensitive test like ELISA or PCR.64 % of children who were negative for rotavirus were breastfed implying that breast feeding was effective in reducing the frequency of Rota virus gastroenteritis.Plenge-Bönig A et al [14] and Indrani Banerjee et al[15] at vellore also reported similar report. This is because human milk contains Human Rota Virus specific IgA antibodies and these antibodies are capable of neutralizing Rota Virus antigens [16].

Out of 100 children included in the study only two had received rotavirus vaccine. As the number of vaccinatated children is negligible, the protective efficacy of vaccine could not be assessed. After RV1 introduction, Mexico saw a 35% reduction in the rate of diarrheal deaths among children [17]. Similar reduction was also reported in Brazil [18].

Active surveillance with sensitive methods like PCR in rotavirus case finding along with further strain typing in an area will help to formulate a vaccine according to the prevalence in that area. Including the vaccine in UIP will significantly reduce the disease burden due to rotavirus infection in children less than 2 years in our country, as India is the leading cause of rotaviral diarrheal diaease in the world.

In conclusion, the present study revealed that rotavirus is the major cause of acute diarrhea in infants and children less than two years of age.So, all children with acute gastroenteritis should have their stool examined for Rota Virus for purpose of correct diagnosis to avoid unnecessary use of antibiotics and emergence of drug resistance. Though ICT has lower sensitivity than PCR, it can be used as an alternate test for rapid diagnosis in situations of emergency testing, as an outpatient procedure. Highly sensitive detection method like RT-PCR is necessary for the purpose of confirmation of diagnosis.

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Table 1- Age wise distribution of rotavirus positive cases

Age group (months)	Positive cases	
	Number	Percentage
0-6	13	20
7-12	39	61
13-18	10	16
19-24	2	3
Total	64	100

Table 2 Comparison of rapid ICT and RT-PCR in detection of rotavirus

Test	Samples tested	Positive		Negative	
		Cases	Percentage	Cases	Percentage
RT-PCR	100	64	64	36	36
ICT		34	34	66	66

Table-3 Evaluation of rapid ICT against RT-PCR

ICT	RT-PCR	
	Positive	Negative
Positive	34	0
Negative	30	36
Total	64	36

Table-4 Association of Breast feeding among positive cases.

Breast feeding	Positive cases		Negative cases		Total	
	No	%	No	%	No	%
Breast fed	27	42	23	64	50	50
Bottle fed	37	58	13	36	50	50
Total	64	100	36	100	100	100

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