Comparision of A Rapid Immunochromatography Test with Elisa to Detect Rotavirus

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Abstract
Introduction: *Worldwide, one of the important causes of childhood hospitalization is diarrhea, with rotavirus being commonly implicated. ELISA is usually used to diagnose the rotavirus infection in microbiology laboratory. It is time consuming and requires sophisticated equipments. We have compared a rapid Immunochromatography test with ELISA in our study. Aim and objective: To detect the presence of rotavirus antigen in the stool sample using ELISA and immunochromatography assay and to know the sensitivity and specificity of Immunochromatography assay compared to ELISA test.*
Materials and Methods: *120 stool samples were collected from the children admitted to the diarrhea ward of Bapuji and Chigateri hospital during June 2010 to May 2011. Stool samples were tested for the presence of rotavirus antigen using ELISA and Immunochromatography assay in the microbiology laboratory at JJM medical college.*
Results: *Of the 120 samples, 24 samples came positive by both ELISA and Immunochromatography assay. However, two samples, which came positive by ELISA, came negative by Immunochromatography. Sensitivity and specificity of Immunochromatography was 91.6% and 97.7% respectively.*
Conclusion: *Immunochromatography test had a good sensitivity and specificity compared to ELISA. Hence it can be used in laboratories with lower burden of samples. It gives quick and relatively precise results.*
Keywords: *Rotavirus, diarrhea, ELISA, Immunochromatography, sensitivity, specificity.*

Introduction

Worldwide, one of the important causes of childhood hospitalization is diarrhea. Children less than five years of age are affected most and one in six children die due to diarrhea. In India approximately 3,34,000 childhood deaths are due to diarrhea [1]. Various viruses, bacteria and protozoa can cause diarrhea. Among them, viruses are commonly implicated in the childhood diarrhea, especially rotavirus [2]. Rotavirus along with norovirus is involved in approximately 40% of all childhood diarrheal cases [3]. Almost all children under five years of age suffer from rotavirus infection once in their lifetime [4].

Rotavirus comes under the genus rotavirus of the family reoviridae. The genome is an eleven-segmented double-stranded RNA. Three concentric layers of proteins surround the genome. The core is made up of viral protein VP2. Middle capsid is represented by VP6. Being most immunogenic, VP6 determines the serogroup of rotavirus. Rotavirus has seven serogroups, A-G.

Serogroup A, B and C have been reported in humans [5]. 30-60% of all severe diarrhea cases are attributable to Group A rotavirus [6].

The mode of transmission is feco-oral route. Few studies have suggested that respiratory droplets can also transmit rotavirus that is swallowed along with mucus, which protects them from acidic content of stomach [5].

Rotavirus infection though seen throughout the year, increases during winter season [7]. Rotavirus gastroenteritis is usually self-limiting, but many a times it leads to severe dehydration due to watery stools and vomiting [5].

Different methods have been developed to diagnose rotavirus infection. It was first observed using electron microscope. Later virus culture, ELISA, latex agglutination, pulse field agarose gel electrophoresis, PCR, immunochromatography test etc. were developed to detect the rotavirus infection. However, commonly used test is ELISA. But it is not feasible for fewer numbers of samples and also takes few hours to give results. Therefore, we aim to study the utility of rapid Immunochromatography (ICG) test and compared it with ELISA.

Materials and Methods

The study was conducted in the Department of Microbiology, J.J.M. Medical College, Davangere, Karnataka during the period from June 2010 to May 2011. One hundred twenty Patients of pediatric age group (six months to five years) having diarrhea (of any duration?) were included in this study. Patients having watery, greenish, foul smelling loose stools were enrolled in this study. Those patients who had blood tinged stools were excluded from the study.

Study design- it is a cross-sectional, hospital-based descriptive study.

Informed consent was taken from all patients' parents/guardians.

Specimen collection

Stool samples from the admitted pediatric diarrhea patients were collected in clean sterile container and were transported to the Microbiology laboratory at the earliest. Samples were stored at -20°C till further processing.

Methodology: In the present study the following methodology was used -

ELISA Method -

100μl of positive control (inactivated simian rotavirus SA-11 in buffered saline with 0.02% thimesoral as a preservative), negative control (sample diluents i.e. buffered saline with 0.02% thimesoral as preservative) and diluted fecal samples, were added to separate wells. 100 μl of enzyme conjugate (Horse radish peroxidase) was added to each well. Contents in the well were mixed and were incubated at room temperature for 60 ± 5 minutes. Wells were washed five times with distilled water. 100 μl of each substrate A (urea peroxide) and B (tetra methyl benzidine) solution was added to each well. These were incubated for 10 minutes at room temperature and 100μl of stop solution (1 N H_2SO_4) was added. Absorbance value for each well was read at 450nm using a > 600nm reference filter against an air blank within 60 minutes.

Specimens with absorbance units (A_{450}) greater than 0.150 were considered positive. Specimens with absorbance value equal or less than 0.150 were considered negative.

Rotavirus Immunochromatography Test (SD bioline)

Preparation of Extracted Sample

1. Test device was allowed to reach room temperature.
2. Assay diluent was taken in a disposable dropper up to the line marked on it and then transferred into the sample collection tube. This was done twice.
3. Sample collection swab was put in to the stool sample and inserted into the tube containing assay diluent.
4. Swab swirled ten times until the sample was dissolved into the assay diluents and swab was discarded while squeezing the swab against the wall of tube.

Test Procedure

1. Test device was removed from the foil pouch and placed on a flat, dry surface.
2. Dropping cap was assembled on the sample collection tube.
3. 4-5 drops of the sample was added to sample well of the test device.
4. As the test began to work, purple color moved across the result window in the center of the test device.
5. Test results were interpreted at 10-20 minutes.

Interpretation of the Test

The presence of only control band within the result window indicates a negative result. The presence of two color bands as test band (T) and control band (C) within the result window, no matter which band appears first, indicates a positive result.

Results

A total of 120 children were included in the study. Age and sex distribution of diarrhea cases is given in table 1. In our study, cases were highest in the age group of six months to two years (20). Among rotavirus positive children, of male children exceeded female (graph1).

Twenty-four children were tested positive for rotavirus by ELISA, giving a prevalence rate of 20% (table 2). ICG also came positive for 24 samples, but few (2) children who were tested positive with ELISA came negative with ICG test (table 3). Also few samples (2), which were reported negative by ELISA, came positive by ICG test. ICG showed a sensitivity of 91.6% and a specificity of 97.9 % when compared with the ELISA (table 4 and 5). Maximum cases were seen during winter months (17 out of 24; 70%) (table 6).

Table-1: Age and Sex Distribution of Diarrhea Cases

Age	Males	%	Females	%	Total	%
6-12 Months	26	21.66	14	11.66	40	33.33
1-2 years	24	20	16	13.33	40	33.33
2-3 Years	14	11.66	8	6.66	22	18.32
3-4 years	6	5.00	4	3.33	10	8.33
4-5 years	4	3.33	4	3.33	8	6.66
Total (6months-5years)	74	61.66	46	38.33	120	

Table-2: Age And Sex Distribution Of Rotavirus Positive Diarrhea Cases By Elisa

Age	Males	%	Females	%	Total	%
6-12 Months	5	20.83	3	12.5	8	33.33
1-2 years	7	29.16	5	20.83	12	49.99
2-3 Years	2	8.33	0	0	2	8.33
3-4 years	1	4.16	0	0	1	4.16
4-5 years	0	0.00	1	4.16	1	4.16
Total (6months-5years)	15	62.5	9	37.5	24	

Table-3: Rotavirus Antigen Detection by Elisa and ICG Methods in Children with Diarrhea in Various Age Groups.

Age	Total No. of cases	ELISA No.(+/-)	ICG No.(+/-)
6-12 months	40	8/ 30	10/30
1-2 year	40	12/20	11/29
2-3year	22	2/20	2/20
3-4 year	10	1/9	1/9
4-5 year	08	1/7	0/8
Total	120	24/96 (20%)	24/96 (20%)

Table-4: Total Number of Positive and Negative Cases Observed with ICG As Compared to Elisa

Rapid Immunochromatography Test (ICG)		ELISA		Total
		Positive	Negative	
	Positive	22	2	24
	Negative	2	94	96
Total		24	96	

Table-5: Diagnostic Efficacy of Immunochromatography Test when Compared with Elisa

Senitivity of immunochromatography test	91.66%
Specificity of immunochromatography test	97.91%
Positive Predictive Value of immunochromatography test	91.66%
Negative Predictive Value of immunochromatography test	97.91%
Accuracy	96.65%

Table-6: Monthly Distribution of Rotavirus Positive Cases

MONTH	NUMBER OF CASES	NUMBER OF ROTAVIRUS POSITIVE CASES	% OF ROTAVIRUS CASES
June	9	0	0
July	10	1	4.17
August	7	1	4.17
September	9	1	4.17
October	13	3	12.50
November	9	3	12.50
December	12	4	16.67
January	16	4	16.67
February	9	3	12.50
March	9	1	4.17
April	9	2	8.33
May	8	1	4.17
Total	120	24	

Out of 24 rotavirus positive diarrhea cases, maximum cases were seen during cooler months (Oct-Feb: 17). Chi-square test = 5.57, p value < 0.05, shows significant association of rotavirus with cooler months than with hotter months

Discussion

Rotavirus usually infects infants and children and is the commonest cause of viral gastroenteritis in them [8].

Various immunological tests are available to diagnose infection caused by rotavirus, of which, commonly used in clinical laboratory is ELISA. ELISA is a very sensitive and accurate test but it requires comparatively costlier apparatus and also skilled technicians to perform the test.

With the availability of rapid immunochromatography assay, diagnosis of rotavirus has become simpler. It is less costly compare to ELISA and does not require any special equipment or skill to perform the assay. Visible bands, which indicate the positivity of the assay can be detected by a laboratory technician easily without the help of a concerned specialist. The test is ideal when few samples are available for testing. Even a single test can be run cost effectively unlike ELISA. ELISA is not cost effective in laboratories getting few samples for rotavirus testing. ELISA takes 3-4 hours to give test result whereas ICG gives results within half an hour [9].

ELISA showed a prevalence of 20% in our study i.e. out of 120 patients, 24 came positive for rotavirus. ICG showed a prevalence rate of 20 %

too. But two cases which came positive with ELISA came negative with ICG.

When ICG was compared with ELISA, it showed a sensitivity of 91.66 % and specificity of 97.91% in our study. Various studies have reported similar results. Momenzadeh A et al did a similar study and compared ICG with ELISA. They found sensitivity and specificity of ICG to be 87.7% and 98.6%, respectively. This is similar to our study [9].

De Rougemont A observed that sensitivity and specificity of immunochromatography test and ELISA were comparable: 96.6% and 96.4%, respectively. The immunochromatography technique was in concordance with the ELISA tests in 93.6% of cases [10]. Another study done by Dennehy PH reported the sensitivity, specificity of ICG as 94% and 100%, respectively. ICG test was reported as a sensitive, specific and relatively simple test [11]. Dewar J et al., found out sensitivity of 88% (66/75) and a specificity of 100% of ICG when compared with the ELISA [12]. Study done by Shaveta D et al showed that ICG is comparable to ELISA with a sensitivity and specificity of 95.24 % and 97.47%, respectively [6].

Regagnon C et al., have reported rotavirus detection by ICG in 30.3- 68% of cases [13].

The present study showed a prevalence rate of rotavirus as 20% in hospitalized children. The prevalence varies from 5 to 71 % [14]. Different studies from different parts of the country supports this, like Chandigarh, Kolkata and Chennai showed a prevalence rate of 16-19%, 5-22% and 20.8%, respectively [6].

Majority of cases were seen in the age group of six months to two years (83.3%). This is the most susceptible age group for rotavirus infection. Infants younger than six months acquire passive immunity from mother, which protects them from infection. This immunity deteriorates with age and infant becomes prone to rotavirus infection by six months of age. Infection rate reduces after two years of age and becomes uncommon after five years of age. This happens as the child acquires active immunity due to repeated rotavirus infection [15]. Raboni SM et al. and Bahl R et al. supported this observation of the present study. They reported that the majority of the cases of rotavirus diarrhea occurred in children younger than 2 years [16, 17].

In comparison to female children, more number of male children were infected with rotavirus. Role of XX chromosomes in providing resistance to infection, has been mentioned for less female infection rate. The gender disparity in orthodox Indian families could also be one of the reasons for male preponderance, where male children are taken to hospital frequently than female children [6]. Other researchers have made similar observation. Banerjee I et al., found out that a larger proportion of children admitted in the hospital due to rotavirus diarrhea were male (63.8%) [18]. In another study done by Junaid SA et al., rotavirus excretion in male and female cases was found 14(8.8%) and 8(5.0%), respectively [19]. Seasonal variation was also seen with rotavirus infection with majority of cases occurred in winter season (October to February). Rotavirus is more stable in the winter months, which facilitates its efficient transmission. It survives for a longer time on the surfaces due to low humidity in the houses [15]. Along with faeco-oral route, spread by droplet infection through respiratory route is also common during winter [6]. This could also contribute to high number of cases during winter season.

Conclusion

We have found out that immunochromatography test can used in place of ELISA in the fields or in case of rotavirus epidemics. ICG assay has a good sensitivity and specificity. With ICG a quick and accurate diagnosis of rotavirus can be made even at the level of primary health centre, thereby providing a rapid and precise treatment approach to handle rotavirus diarrhea patient.

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