Immunochromatographic Test and ELISA for Detection of Rotavirus in Fecal Sample: A Comparative Study

Sanjoy Kanti Biswas1*
Nahid Sultana1
Md. Badruddoza2
Md. Anwarul Azim2
Rajat Sankar Roy Biswas3

1Department of Microbiology
Chattogram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.
2Department of Paediatrics
Chattogram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.
3Department of Medicine
Chattogram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.

*Correspondence to:
Dr. Sanjoy Kanti Biswas
Professor
Department of Microbiology
Chattogram Maa-O-Shishu Hospital Medical College
Chattogram, Bangladesh.
Mobile: +88 01715 63 90 17
Email: sanjoyustc@gmail.com
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Abstract
Background: Rotavirus is a common cause of diarrhea in children. Early diagnosis of rotavirus infection helps to determine appropriate treatment thus preventing unnecessary use of antibiotics and minimizes its spread. Rapid test using lateral flow immunochromatography is a good alternative to Enzyme Linked Immunosorbent Assay (ELISA) with good sensitivity. This study was undertaken to compare Immunochromatographic Test (ICT) with ELISA for detection of rotavirus antigen in stool sample.

Materials and methods: Ninety stool samples from hospitalized children less than two years of age with symptoms of acute gastroenteritis were tested by both ICT and ELISA. Sensitivity and specificity was compared.

Results: Out of 90 samples 63.3% were positive for rotavirus antigen by ELISA and 51% were positive by ICT. Sensitivity and specificity of ICT was determined considering ELISA as a gold standard. Sensitivity of ICT was 80.7% whereas specificity was 100%.

Conclusion: Rotavirus gastroenteritis cases can be diagnosed by rapid test (Immunochromatography) with high sensitivity and specificity.

Key words: Rotavirus; ELISA; ICT; Acute gastroenteritis.

INTRODUCTION
Rotavirus is the leading cause of severe childhood gastroenteritis in both developed and developing countries. Worldwide >125 million infants and young children develop rotavirus-associated diarrhea each year, resulting in 4,40,000 infant and child deaths, mostly in developing countries. In Bangladesh, rotaviruses cause 6,000–14,000 deaths each year in children <5 years of age1. It has been estimated that 29% of all diarrheal deaths in children <5 years of age are due to Rota virus and about 23% of them are in the Indian subcontinent2. Approximately 95% of children are infected before 3–5 years of age, and the highest incidence occurs between 6 and 24 months of age3.

The rotavirus belongs to the Reoviridae family, exhibits icosahedral symmetry, non-enveloped and was first identified by electron microscopy by Bishop et al. The capsid consists of three layers of protein and the viral genome consists of 11 segments of double-stranded RNA (dsRNA) which encode six structural proteins, VP1-4, VP6 and VP7 and six non-structural proteins, NSP1-6. The VP6 protein, located in the inner layer of capsid of the virus contains the antigenic determinants, which allow their classification into seven serogroups of A to G, with group A being the most common agent of childhood diarrhea4,5. Early diagnosis of rotavirus infection prevents unnecessary use of antibiotics, minimizes its spread and helps to determine the appropriate treatment.
There are various methods for detection of rotavirus from stool specimen like, Enzyme Immunoassay [EIA] Latex agglutination, Lateral flow immuno- chromatography, RT-PCR and electron microscopy. Although highly specific, electron microscopy is too labour intensive and expensive. RT-PCR is highly sensitive and specific but it is expensive, labour intensive and highly trained staff is required. Hence both techniques are not suitable for routine diagnosis. The Enzyme Immunoassay [EIA] is highly sensitive but it requires expertise and well established laboratory set up. Rapid test using latex agglutination or lateral flow immunochromatography is a good alternative to EIA with good sensitivity. Results of rapid test are available within 15 to 20 minutes while EIA takes 3 to 5 hours. Rapid test can also be done bed side; it does not require much expertise. Hence, the present study was undertaken to compare rapid Immunochromatography (ICT) method with Enzyme Linked Immunosorbent Assay (ELISA) for detection of rotavirus antigen in stool sample in our settings.

MATERIALS AND METHODS
This cross sectional study was conducted in the Microbiology Department and Paediatric Department of Chattogram Maa-O-Shishu Hospital Medical College (CMOSHMC) during the period of 1st September 2018 to 15th December 2018. Ninety children less than two years old with acute gastroenteritis, who were admitted into the diarrheal unit of Paediatric Department, were included in the study. The exclusion criteria were chronic diarrhea (Which was defined as diarrhea that lasted for more than two weeks) diarrhea stool mixed with blood and mucus. Written informed consent for participation was obtained from parents/guardians of the children. We obtained the Institutional Review Board’s approval for the study before it was initiated.

Fresh stool samples were obtained within 24 to 48 hours of admission. Sterile wide-necked plastic containers were used to collect and transport the samples. Macroscopic examination of the samples was conducted as follows: color, consistency, presence of blood, presence of mucus, presence of worms. Microscopic examination were also performed to see the presence of pus cells and RBCs. The labeled stool samples were stored at -20°C until they were assayed.

Detection of rotavirus by Immunochromatography Test (ICT) - ICT Quick Rotavirus kits (Arco Biotech, Germany) were used to detect rotavirus antigen in stool samples. This test is a single-step, immunochromatographic lateral flow test and it was conducted according to the manufacturer’s instructions. Two bands should appear to indicate rotavirus positive; the control band and test band are visible. If only control band is visible, it is rotavirus negative. If control band is missing, the test is invalid.

Detection of rotavirus by ELISA - EDI™ Fecal Rotavirus Antigen ELISA Kit (Eptope Diagnostics, Inc. San Diego, CA 92121, USA) was used to detect rotavirus antigen in stool samples. In this test, monoclonal antibodies against the product of sixth viral gene (VP6) were used in a sandwich-type method. The assay was conducted according to manufacturer’s instructions.

RESULTS
90 children below two years of age with acute gastroenteritis were screened for Rotavirus antigen. Of the 90 patients, 46 (51.1%) were males. Of this, 28 (31.1%) were in the age group of 7-12 months and 09 (10%) were in the 13-18 months of age. The remaining 44 (48.9%) were female children. Of this 24 (26.7%) were in the age group of 7-12 months and 12 (13.3%) were in the age group of 13-18 months (Table I).

Table I: Age and gender distribution of study group (n=90)

| Age (Months) | Male | Female | Total |
|--------------|------|--------|-------|
| 0 – 6        | 08 (8.9%) | 05 (5.6%) | 13 (14.5%) |
| 7 – 12       | 28 (31.1%) | 24 (26.7%) | 52 (57.8%) |
| 13 – 18      | 07 (10%) | 12 (13.3%) | 21 (23.3%) |
| 19 – 24      | 01 (1.1%) | 03 (3.3%) | 04 (4.4%) |
| Total        | 46 (51.1%) | 44 (48.9%) | 90 (100%) |

Table II: Rotavirus positive gastroenteritis cases observed by ELISA and ICT

| Test | No. of specimen | Positive | Negative |
|------|----------------|----------|----------|
| ELISA | 57 (63.3%) | 33 (36.7%) |
| ICT  | 46 (51.1%) | 44 (48.9%) |

All the 90 samples were tested by both ELISA and rapid ICT for rotavirus in their stool samples. Out of this ELISA was positive for 57 (63.3%) of samples while 46 (51.1%) were positive by ICT as shown in Table II.

Table III: Age distribution of ELISA positive rotavirus gastroenteritis cases (n=57)

| Age (Months) | Number | Percentage |
|--------------|--------|------------|
| 0 – 6        | 07     | 12.3       |
| 7 – 12       | 34     | 59.7       |
| 13 – 18      | 14     | 24.5       |
| 19 – 24      | 02     | 3.5        |
| Total        | 57     | 100        |

Table III shows the maximum number of Rotavirus positivity was seen in the age group of 7 to 12 months (59.7%) followed by 13 to 18 months (24.5%). The infection rate was found to be lower in the age group of 0 to 6 months (12.3%); likewise after 18 months of age, Rotavirus infection decreases sharply (3.5%).
Table IV: Spectrum of clinical manifestations among positive Rotavirus and negative gastroenteritis cases

| Clinical manifestations | Rotavirus positive patients (n=57) | Rotavirus negative patients (n=33) |
|-------------------------|-----------------------------------|----------------------------------|
| Fever                   | 21 (36.8%)                        | 11 (33.3%)                      |
| Vomiting                | 36 (63.2%)                        | 19 (57.6%)                      |
| Dehydration             |                                   |                                 |
| None                    | 4 (7.0%)                          | 4 (12.1%)                       |
| Mild to moderate        | 48 (84.2%)                        | 25 (75.8%)                      |
| Severe Dehydration      | 5 (8.8%)                          | 4 (12.1%)                       |
| Rehydration             |                                   |                                 |
| Oral                    | 32 (56.1%)                        | 11 (33.3%)                      |
| Intravenous             | 25 (43.9%)                        | 22 (66.7%)                      |

Table IV shows that fever and vomiting were present in 36.8% and 63.2% cases respectively among rotavirus positive gastroenteritis patients. Mild to moderate dehydration were present in 84% cases of rotavirus positive gastroenteritis and 56% were treated by oral rehydration therapy and 66.7% of rotavirus negative gastroenteritis cases required intravenous rehydration.

Table V: Socio-demographic data

| Characteristics      | Total no. of cases (n=90) | Rotavirus positive gastroenteritis (n=57) | Rotavirus negative gastroenteritis (n=33) |
|----------------------|--------------------------|-----------------------------------------|-----------------------------------------|
| Gender               |                          |                                         |                                         |
| Male                 | 46 (51.1%)               | 31 (54.4%)                              | 15 (45.5%)                              |
| Female               | 44 (48.9%)               | 26 (45.6%)                              | 18 (54.5%)                              |
| Water supply         |                          |                                         |                                         |
| Public water         | 55 (61.1%)               | 35 (61.4%)                              | 20 (60.6%)                              |
| Bore well water      | 35 (38.9%)               | 22 (38.6%)                              | 13 (39.4%)                              |
| Geography            |                          |                                         |                                         |
| Urban                | 67 (74.4%)               | 43 (75.4%)                              | 24 (72.7%)                              |
| Rural                | 23 (25.6%)               | 14 (24.6%)                              | 9 (27.3%)                               |
| Socioeconomic status of parents |        |                                         |                                         |
| Low                  | 47 (52.2%)               | 31 (54.4%)                              | 16 (48.5%)                              |
| Middle class         | 37 (41.1%)               | 23 (40.4%)                              | 14 (42.2%)                              |
| High                 | 6 (6.7%)                 | 3 (5.2%)                                | 3 (9.3%)                                |
| Maternal education   |                          |                                         |                                         |
| Less than high school | 26 (28.9%)               | 18 (31.6%)                              | 8 (24.2%)                               |
| High school          | 40 (44.4%)               | 26 (45.6%)                              | 14 (42.4%)                              |
| More than high school | 24 (26.7%)               | 13 (22.8%)                              | 11 (33.4%)                              |
| Feeding              |                          |                                         |                                         |
| Breast milk          | 17 (18.9%)               | 8 (14%)                                 | 9 (27.3%)                               |
| Mixed                | 83 (91.1%)               | 49 (86%)                                | 24 (72.7%)                              |
| Rotavirus vaccine    |                          |                                         |                                         |
| Received             | 3 (3.3%)                 | 0                                       | 3 (9.1%)                                |
| Not received         | 87 (96.7%)               | 57 (100%)                               | 30 (90.9%)                              |

In this study, out of 90 children 57 were positive for Rotavirus antigen; of which male children showed 54.4% (31/57) and female children showed 45.6% (26/57) positivity. Higher incidence of Rotavirus positive cases were found in those using public water supply (61.4%) than those used bore well water (38.6%). Despite Rotavirus being prevalent in all geographical areas, 74.4% of cases were from urban whereas 25.6% from rural areas, were found in this study.

Poor literacy of mother is linked with increased rate of diarrheal diseases in young children. In this study 54.4% of Rotavirus infected children belongs to low socioeconomic group and 40.4% from middle class. It shows low socioeconomic status has been linked with higher incidence of rotavirus gastroenteritis. In this study, lower incidence of rotavirus gastroenteritis (14%) were seen among children who were on exclusive breast feeding. 100% rotavirus positive gastroenteritis cases did not receive rotavirus vaccine (Table V).

Table VIa: Comparison of ICT and ELISA test for the detection of rotavirus antigen from stool sample (n=90)

| ICT                  | Positive | ELISA      | Total |
|----------------------|----------|------------|-------|
| Positive             | 46 (51.1%)| 0 (0%)     | 46 (51.1%)|
| Negative             | 11 (12.1%)| 33 (36.7%) | 44 (48.9%)|
| Total                | 57 (63.3%)| 33 (36.7%) | 90 (100%)|

51% cases were positive for rotavirus antigen by both ICT and ELISA and 12% cases were positive for rotavirus antigen by ELISA but negative by ICT. No ELISA negative cases were found to be positive by ICT and 36.7% cases were negative for rotavirus antigen by both ICT and ELISA (Table VIa).

Table VIb: Diagnostic efficacy of ICT as compared to ELISA test.

|                      | ICT Positive | ELISA Negative | Total |
|----------------------|--------------|----------------|-------|
| True positive        | 46           | 0              | 46    |
| True negative        | 33           |                | 33    |
| False positive       | 0            |                | 0     |
| False negative       | 11           |                | 11    |
| Sensitivity of ICT   | 80.7%        |                |       |
| Specificity of ICT   | 100%         |                |       |
| Positive predictive value | 100%    |                |       |
| Negative predictive value | 75%    |                |       |

Sensitivity of rapid ICT was 80.7% and specificity was 100% when evaluated against ELISA, a gold standard test. Positive predictive value of ICT was also 100% and Negative predictive value of ICT was 75% (Table VIb).

DISCUSSION

This study attempts to evaluate the rapid immunochromatographic test against ELISA in diagnosing rotavirus in stool samples of children less than two years of age. Stool samples were collected from 90 hospitalized children of less than 2
years of age with diarrhea. Around half of the patients (57.8\%) in the study group were between 7-12 months of age. This was similar to a study by Calistus Wilunda et al\(^9\). Higher risk of diarrhea in children of this age group could be related to beginning of environmental exposure and weaning of children who have immature immune system. Male (51\%) and female (49\%) children in the study group were equally affected by acute diarrheal diseases. Gender difference was not associated with the incidence of acute diarrhea which was similar to a study by Ali Aghhar Kolahi et al, since boys and girls were probably equally exposed to the risk factors with acute diarrhea which may be related to the environment, socio-demographic status and other biological agents\(^10\).

In the present study, Rota virus accounts for 63.3\% of hospitalization in children with acute diarrheal illness. Similarly Satter et al from Bangladesh in 2017 found 64\% rotavirus positive gastroenteritis cases among hospitalized children\(^11\). The prevalence is comparable to a study conducted by Hamsa T Tayeb et al in 2011\(^12\). Global prevalence of Rotavirus gastroenteritis in children below 5 years varies from 6\% to 56\%, whereas in India the incidence of Rotavirus diarrhoea is from 5\% to 89.9\%\(^12\). The wide difference in the prevalence of Rotavirus gastroenteritis may be due to difference in the age group, different diagnostic methods conducted, time of onset of disease and seasonal variation of Rotavirus and differences in geographical distribution of Rotavirus among various countries.

In this study, the peak incidence of Rotavirus occurred in the age group of 7 to 12 months (59.7\%) which is closely related with the study by John et al who reported 78.3\% Rotavirus positive cases in the age group between 6 to 15 months\(^2\). The higher frequency of Rotavirus diarrhea in this age group is because of weaning from breast feed and introduction of artificial feeds. The chance of infection tends to increase when breast feed is withdrawn since the protective antibodies are lost. Furthermore, introduction of artificial feed is linked with increased risk of infection unless proper hygienic measures are strictly followed. Lesser frequency of Rotavirus incidence was observed in the babies of 0 to 6 months (12.3\%) of age which may be due to protection offered by maternal antibodies against Rotavirus infection. The occurrence of Rotavirus declined sharply after 18 months of age (3.5\%). This is because of subsequent infections that usually occur less severely due to the antibodies formed against previous exposure to Rotavirus.

Diarrhea, dehydration, vomiting and fever are the common symptoms observed in Rotavirus infected children. In our study, vomiting was noted in 63.2 \% of Rotavirus positive children. Mild to moderate dehydration was found in 84.2\% of Rotavirus positive cases. Fever was found more or less equally in both Rotavirus positive and negative cases. The above findings from this study are consistent with a study done by Nymbat et al in Srilanka\(^13\). Regarding management, Rotavirus positive cases need supportive measures which include prompt correction of fluid and electrolyte imbalance by ORS/IVF.

In this study out of 90 children, 57 were positive for Rotavirus antigen of which male children showed 54.4\% (31/57) and female 45.6\% (26/57) positivity. The present study showed that male children were more susceptible for Rotavirus infection than female children (M:F - 1.2:1) which correlates with the study by Mehta et al., from India reported a ratio of 1.6:1 in male and female children\(^8\).

Despite Rotavirus being prevalent in all geographical areas, in this study the positivity rate is higher in urban (75.4\%) than rural areas. This may be due to early weaning from breast feeding and introduction of artificial feeds and increasing day care centers where subclinical infections of Rotavirus occur throughout the year and transmission through fomites is also a possibility.

There was a higher frequency of Rotavirus infection in those using public water (61.4\%) supplies than those who used bore wells (38.6\%) as evidenced in this study. Similarly Tate J et al found rotavirus gastroenteritis in 54\% children who use public water\(^14\). Group A Rotavirus is especially known to cause outbreaks of water borne acute gastroenteritis in children and adults in China, India, Bangladesh and Myanmar\(^15\).

Literacy of the mother also reflects on the health of the children as indicated in our study. Children whose mother had only primary and secondary level education seem to be affected more by Rotavirus infections (77\%). This could be due to lack of knowledge regarding personal and general hygienic measures as well as teaching hygienic practices to their children\(^16\).

In this study, lower incidence of rotavirus gastroenteritis was seen among children (14\%) who were exclusively breast feed than the children on mixed feed (86\%). This may be due to protection afforded by maternal IgA anti Rotavirus antibodies\(^17\). In addition, introduction of supplementary feed increases the chance of infection considerably unless proper hygienic measures such as proper sterilization of feeding bottles/bowls and washing hands with soap and water before preparing/feeding food are strictly followed.

Out of 90 children included in the study only three had received rotavirus vaccine. As the number of vaccinated children is negligible, the protective efficacy of vaccine could not be assessed. After RV1 introduction in Brazil in 2006, 30\% and 39\% decreases in gastroenteritis mortality were noted in 2007 and 2008, respectively, when compared to the mortality rates in 2004-2005\(^18\).

In this study sensitivity of the ICT was 80.7\% and specificity was 100\% considering ELISA as gold standard. In Dhiman et al study, sensitivity of rapid test was 95.24\% and specificity was 97.47\%\(^19\). Ibrahim et al found sensitivity and specificity of rapid test (immunochromatography) as 90\% and 100\% respectively\(^20\). These findings are co-relating well with present study.

CONCLUSION

Rotavirus is an important cause of acute gastroenteritis in children less than two years of age. Its delayed diagnosis can lead to higher morbidity and mortality. Its early bed side diagnosis can be done by rapid test (Lateral flow immunochromatography) with high sensitivity (80.7\%) and specificity (100\%).
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DISCLOSURE
All the authors declared no competing interest.

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