Original Research Article

Comparative study of enzyme linked immunosorbent assay and immunochromatography for rotavirus detection in children below five years with acute diarrhoea

Mehta N[1], Baveja S[2*]

[1]Senior Resident, Department of Microbiology, ESI - PGIMSR , Central road,MIDC, Andheri (East), Mumbai 400093, Maharashtra, INDIA.
[2]Professor and HOD, Department of Microbiology, LTMMC&GH, Dr. Babasaheb Ambedkar Road, Sion (West), Mumbai, 400022, Maharashtra, INDIA.
Email: nehalashokmehta@gmail.com

Abstract

Background: Early diagnosis of rotavirus infection prevents unnecessary use of antibiotics, minimizes it's spread and helps to determine the appropriate treatment. Rapid test using latex agglutination or lateral flow immunochromatography is a good alternative to EIA with good sensitivity. The present study was undertaken to compare rapid immunochromatography method with Enzyme linked immunosorbent assay (ELISA) for detection of rotavirus antigen in stool sample. **Material and Methods:** Two hundred stool samples fromhospitalized children less than 5 years of age with symptoms of acute gastroenteritis were tested by rapid SD Bioline test and ELISA Kit (Premier™Rotaclone®). Sensitivity and specificity was compared. **Results:** All 200 stool specimens were processed for rotavirus antigen by rapid test and ELISA, out of which 26% were positive by rapid test and 28% were positive by ELISA. Sensitivity and specificity of rapid kit was studied considering ELISA as a gold standard. Sensitivity of rapid test was 89.29% whereas specificity was 98.6%. **Discussion:** Its early bed side diagnosis can be done by rapid test (lateral flow immunochromatography) with high sensitivity (89.29%) and specificity (98.6%).
Key Words: Rotavirus, ELISA, Rapid immunochromatography test, acute diarrhoea.

***Address for Correspondence:**
Dr. Baveja S, Professor and HOD, Department of Microbiology, LTMMC&GH, Dr. Babasaheb Ambedkar Road, Sion (West), Mumbai, 400022, Maharashtra, INDIA.
Email: nehalashokmehta@gmail.com
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INTRODUCTION

In India, diarrheal disease is an important public health problem among under-five children. Diarrhea in children can be due to various causes like viruses, bacteria, protozoa and helminths. Among viral causes, rotavirus was most common cause of acute gastroenteritis in children under five years of age. They are responsible for 30 to 60% of all cases of severe watery diarrhea in young children[1]. In India, rotavirus is estimated to cause 34% of all diarrheal death in children[2]. Early diagnosis of rotavirus infection prevents unnecessary use of antibiotics, minimizes it's spread and helps to determine the appropriate treatment[3]. There are various methods for detection of rotavirus from stool specimen like, Enzyme immunoassay [EIA], Latex agglutination, Lateral flow immune- chromatography, RT-PCR and electron microscopy[4]. 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 is

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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. RT-PCR is highly sensitive and specific but it is expensive, labour intensive and highly trained staff is required, hence not suitable for routine diagnosis. Although highly specific, electron microscopy is too labour intensive, expensive and not suitable for routine diagnosis. Hence, the present study was undertaken to compare rapid immunochromatography method with Enzyme linked immunosorbent assay (ELISA) for detection of rotavirus antigen in stool sample.

MATERIAL AND METHODS

This prospective study was conducted over a period of one year from January 2014 to December 2014 in a Department of Microbiology at a tertiary care hospital. Two hundred stool samples from hospitalized children less than 5 years of age with symptoms of acute gastroenteritis were included after obtaining approval from the institutional ethics committee. Children above five years of age, outdoor patients and not giving consent were excluded from study. Consent was taken from the guardian of child for participation in the study after explaining the protocol to the guardian in the language that the guardian best understood. A detailed history including the demographic profile, presenting complaints, past history and risk factor was elicited for each patient and duly recorded in the case record forms. Freshly passed (preferably on the day of presentation itself), 3 to 5 ml of stool samples were collected in a dry, sterile, wide mouth container from hospitalized children with acute gastroenteritis by the help of their guardian and transported to Microbiology laboratory immediately. Macroscopic examination of stool specimen was done and then it was divided in threeparts. One part was processed for microscopic examination and culture. Second part was used for rapid test to detect rotavirus antigen from stool specimen. Third part was stored at -20ºC to perform rotavirus antigen Enzyme linked immunosorbent assay [ELISA] subsequently. Second part of the stool specimen was used to detect rotavirus antigen by rapid SD Biolinetest. The test cassette has letter 'T' as Test line and 'C' as Control line on the surface of the device. All two lines in result window are not visible before applying any samples. A purple test line will be visible in result window if there are enough rotaviruses in sample. If rotavirus is not present in sample, there is no color appearance in 'T'. Third portion of stool specimen was stored at -20ºCfor ELISA Kit (Premier™ Rotaclone®). An aliquot of faecal suspension is added to the well and incubated simultaneously with an anti-rotavirus monoclonal antibody conjugated to horseradish peroxidase. After 60 minutes of incubation at room temperature, the sample well is washed. Enzyme substrate A. (urea peroxide) and substrate B (TMB) are added to the well and incubated for 10 minutes at room temperature. The enzyme bound in well converts the colorless substrate to blue color. The intensity of blue color is directly proportional to the concentration of viral particle in stool sample. Premier™ Rotaclone® contains a positive control and negative control (sample diluent) which was run with each assay. By spectrophotometric determination method, specimens with absorbance units (A450) greater than 0.150 were considered positive and absorbance units (A450) equal to or less than 0.150 were considered negative. All results were noted down on case record form of respective patients and results were analyzed.

RESULTS

A total of 200 hospitalized children below five years of age with acute gastroenteritis were studied for rotavirus as a cause of diarrhea. Out of 200 patients, 61.5% were male and 38.5% were female. Male: Female was 1.6:1.Maximum cases of acute gastroenteritis belonged to age group 6 months to 2 years (53.5%) followed by more than 1 month to six months of age (27.5%),2 year to 5 years (14.5),≤ 1 month (4.5%). Out of 200 samples 52 were positive for rotavirus antigen by rapid test, 56 were positive by ELISA test.

Table 1: Positive and negative cases observed with Rapid and ELISA test

Test	Positive	Negative	Total
Rapid test	52 (26%)	148 (74%)	200
ELISA test	56 (28%)	144 (72%)	200

Out of 200 cases of acute gastroenteritis, 52 were positive by rapid test and 56 were positive by ELISA for rotavirus antigen.

Table 2: Comparison of Rapid and ELISA test for detection of Rotavirus antigen from stool sample (n=200)

Rapid test	ELISA				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
Positive	50	25	02	01	52	26
Negative	06	03	142	71	148	74
Total	**56**	**28**	**144**	**72**	**200**	**100**

25% of cases were positive for rotavirus antigen by both rapid test and ELISA. 3% of cases were positive for rotavirus antigen by ELISA but negative by rapid test. 1% of case was positive for rotavirus antigen by rapid test but negative by ELISA. 71% of cases were negative for rotavirus antigen by both rapid test and ELISA. Sensitivity and specificity of rapid kit was studied considering ELISA as a gold standard.

Table 3: Diagnostic efficacy of Rapid test as compared to ELISA test

True positive	50
True negative	142
False positive	02
False negative	06
Sensitivity of rapid test (%)	89.29
Specificity of rapid test (%)	98.6
Positive predictive value (%)	96.15
Negative predictive value (%)	95.95

DISCUSSION

Rotavirus is most common cause of severe diarrheal disease in infants and children globally. Rotaviruses are estimated to be responsible for approximately 527,000 deaths in children, with more than 85% of these deaths occurring in low income countries in Asia and Africa. Over two million children are hospitalized each year with pronounced dehydration because of rotavirus[5]. Therefore, it is important to diagnose it early in patients of acute gastroenteritis so that appropriate treatment can be started and unnecessary use of antibiotic can be avoided. Several methods are available for detecting rotavirus in stool specimen which includes rapid tests like latex agglutination and lateral flow immunochromatography, Enzyme linked immunosorbent assay (ELISA), RT-PCR, electron microscopy etc. Rapid tests can be performed easily at bed side of patient, it doesn't need much expertise and results are available within few minutes. ELISA can be used in routine laboratory to detect rotavirus antigen in stool. Large number of samples can be tested at a time in 96 well plate in few hours. RT-PCR and electron microscopy needs expertise, expensive and not suitable for routine diagnosis of rotavirus.[6] It is used only for epidemiological and research purpose. In present study, total 200 hospitalized children less than 5 years of age with acute gastroenteritis were studied. From all 200 cases of acute gastroenteritis stool specimen was collected which was tested for rotavirus antigen by rapid test (lateral flow immunochromatography) and ELISA. In present study, ELISA was considered as gold standard and sensitivity, specificity of rapid test was observed. In present study rotavirus antigen was detected by both, rapid test and ELISA. ELISA was considered as gold standard. Out of 200 stool specimens, 26% were positive by rapid test and 28% were positive by ELISA for rotavirus antigen. (Table 1). There are various studies which showed similar finding to present study as shown in following table.

Table 4: Comparison of percentage positivity of rotavirus diarrhea reported by various studies

Sr.	Author	Year	Place	Total no. of study cases	% positivity of rotavirus
1	DhimanS *et al*[10]	2015	Amritsar	100	21%
2	BadurM *et al*[8]	2015	Tirupati	187	25.67%
3	MullickS *et al*[9]	2014	Kolkata	1568	25.2%
4	SherchandJ *et al*[11]	2009-11	Nepal	2718	24%
5	BehlR *et al*[12]	2001	New Delhi	584	23.5%
6	Present study	2015	Mumbai	200	28%

The rapid test can be conducted on bed side and it does not require much expertise hence in present study rapid test was compared with ELISA. In present study sensitivity of rapid test was 89.29% and specificity of rapid test was 98.6% (Table 3). In Shaveta Dhiman *et al*[10] study, sensitivity of rapid test was 95.24% and specificity was 97.47%. Salwa Badrelsabbah Ibrahim *et al*[13] found sensitivity and specificity of rapid test (immunochromatography) as 90% and 100% respectively. These findings are co-relating well with present study. To conclude, rotavirus is important cause of acute gastroenteritis in children less than five 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 (89.29%) and specificity (98.6%).

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