European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 9, Issue 6, 2022

ORIGINAL RESEARCH

A Comparative Evaluation Of Two Different Rapid Tests With ELISA To Detect Rotavirus In Paediatric Stool Samples

[1]Ankita Gupta, [2]Mamta Sharma, [3]Imsensenba Longkumer, [4]Archana Sharma

[1]Assistant Professor, Department of Microbiology, Santosh Medical College, Ghaziabad, Uttar Pradesh, India
[2,4]Associate Professor, [3]Assistant Professor, Department of Microbiology, Al-Falah School of Medical Science and Research Centre and Hospital, Faridabad, Haryana, India

Corresponding author
Dr. Archana Sharma
Associate Professor, Department of Microbiology, Al-Falah School of Medical Science and Research Centre and Hospital, Faridabad, Haryana, India
Email: dr.archana82@gmail.com

Received: 02 June, 2022 Accepted: 05 July, 2022

ABSTRACT

Background and objectives: Rotaviruses are the major cause of gastroenteritis in infants and young children worldwide. The mortality and economic burden associated with rotavirus is high especially in developing countries. ELISA is most commonly used for detection of rotavirus. In this study we have compared the results of two rapid and simple tests with ELISA.

Materials and methods: The study was conducted in the Department of Microbiology, J.J.M. Medical College, Davangere (June 2010-May 2011). The study included 120 stool samples from pediatric patients of age group 6 months to 5 years. The samples were tested for the presence of rotavirus antigen using Enzyme linked immunosorbent assay (ELISA), latex agglutination (LA) and immunochromatography (ICG) test.

Results: Prevalence of Rotavirus diarrhea was found to be 20% in children using ELISA with maximum cases seen in age group of 6 months to 2 years (83.3%) with male preponderance. Rotavirus infection was seen throughout the year with a peak in cases during the months of October to February. In present study latex agglutination test was found to be more sensitive than Immunochromatography. However, ICG was found to be more specific than LA.

Conclusion: Rotavirus infection was seen in a considerable proportion of infants and children in present study. Latex agglutination and immunochromatography test were found to have good sensitivity and specificity, when compared with ELISA, for detection of rotavirus antigen. These tests can be used for screening cases of suspected rotavirus diarrhea during an epidemic outbreak. Rapid and accurate diagnosis of rotavirus diarrhea is important to avoid unnecessary administration of antibiotics in children and to prevent spread of infection to other children in hospital and community.

Key words: Rotavirus; Enzyme Linked Immunosorbent Assay; Latex Agglutination; Immunochromatography Test.

INTRODUCTION

Among the major public health diseases that lead to significant morbidity and mortality, acute

diarrheal disease plays an important role, both in developing and developed countries.[1]
Approximately 800000 children of age less than five years old have been reported to succumb to diarrhoea annually. Various factors like unclean water, contaminated food, poor hygiene, inadequate disposal of waste and faeces were associated with such a high mortality due to diarrhoea, however the most common cause was found to be viral infection.[2]
The major cause of acute severe dehydrating diarrhoea in young children is Rotavirus.[1,5] Clinical manifestations of rotavirus gastroenteritis includes profuse diarrhea, mild fever, and vomiting, leading to mild to severe dehydration. Diagnosis of rotavirus gastroenteritis cannot be decided solely on clinical features and laboratory tests are required to confirm its diagnosis.[3,4]
Rotavirus is a spherical, non-enveloped RNA virus that has derived its name from the Latin word rota, meaning wheel. It comes under *Reoviridae* family and consists of core, inner capsid and the external capsid.[6] Its genome has 11 double stranded ribonucleic acid segments those codes for twelve different proteins.[7] Out of these proteins, NSP4 is important. It's a diarrhoea inducing enterotoxin. The most immunogenic protein is VP6 protein. Based on VP6 protein rotavirus is divided into eight groups i.e. A-H. The most common group in humans is Group A.[6]RT-PCR and ELISA are the standard test for diagnosing rotavirus gastroenteritis. Various rapid serological tests like latex agglutination test and immunochromatographic test, have been evaluated and compared toother methods, such as ELISA and quantitative real time RT- PCR (qr RT-PCR), and they have shown a wide range of sensitivity and specificity.[8] Since ELISA is time consuming and is suitable for large number of samples, we have compared immunochromatographic test and latex agglutination assay with ELISA as they are suitable for even a single sample and are time saving.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, J.J.M. Medical College, Davengere, Karnataka, during the period from June 2010 to May 2011. It was a cross-sectional, hospital based descriptive study. Total 120 pediatric patients of age group between six months to five years, having diarrhea were included in this study. Patients who had foul smelling, greenish watery stools were enrolled in this study and patients with blood-tinged stools were excluded from the study. Prior to the study informed consent was taken from all patients' parents/guardians.

SPECIMEN COLLECTION

Once the patient gets admitted to the pediatric diarrhea ward, the parents were given a clean container to collect the stool sample. From there, the sample was transported to the Microbiology laboratory at the earliest where they were stored at -20° C till further processing.
Rotavirus antigen was detected in fecal samples using enzyme linked immunosorbant assay (ELISA), Latex Agglutination assay and Immunochromatography test.
ELISA was performed using Rotaclone kit as per the manufacturers' instructions. Briefly the procedure is as follows-

SPECIMEN PREPARATION

1 ml of sample diluent (buffered saline with 0.02% thimerosal as preservative) was added in a tube with a micropipette. Then the stool sample was added to the diluent with the help of a transfer pipette.

PROCEDURE

100µl of diluted fecal sample, positive control (inactivated simian rotavirus SA- 11 in

buffered saline with 0.02% thiomersal as a preservative) and negative control (sample diluents i.e., buffered saline with 0.02% thiomersal as preservative) were added to the bottom of separate wells. Then 100 µl of enzyme conjugate was added to each well. Contents in well were mixed well by gently swirling on table top followed by incubation at room temperature for one hour. Wells were washed five times with distilled water. 100 µl of each substrate A (urea peroxide) and B (tetra methyl benzidine) solution were added to each well. Wells were incubated for 10 minutes at room temperature and 100µl of stop solution (1 N H2SO4) was added to each well.

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 (A450) greater than 0.150 were considered positive. Specimens with absorbance value equal to or less than 0.150 were considered negative.

Latex Agglutination assay was carried on each sample using the Plasmatec Rota Virus Latex Agglutination kit. Manufacturer's instructions were followed while performing test. A positive result was indicated by the visible agglutination of the test latex particles and negative result was indicated by a milky appearance without any visible aggregation of the latex particles.

Rotavirus Immunochromatographic Test was performed using SD BIOLINE (One step Rotavirus Antigen Test) kit as per the instruction given in the kit. The presence of both control band and test band within the result window indicates a positive result whereas formation of only control band indicates negative result.

RESULTS

This study included 120 children suffering from diarrhea in age group of 6 months to 5 years. Rotavirus antigen was detected in 24 stool samples by ELISA. Maximum cases were seen in the age group of 6 months to 2 years (20), which was statistically significant (Chi-square test = 7.86, p value < 0.05).

There were 74 male and 46 female children in the study. Out of them number of rotavirus positive male and female children were 15 and 9, respectively.

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.

[Table1] shows the distribution of diarrhea cases and rotavirus positive cases according to age and sex. [Table2] shows month wise distribution of diarrhea cases and rotavirus positive children.

With Latex agglutination assay and Immunochromatography test, 26 and 24 samples came positive respectively. Table3 shows detection of Rotavirus Antigen by Different Methods in children with Diarrhea in Various Age Groups.

ELISA- Enzyme linked immunosorbent assay LA- Latex agglutination ICG- Immunochromatography

When compared to standard test- ELISA, Latex agglutination showed a sensitivity of 95.8% and specificity of 96.87% whereas immunochromatography test showed a sensitivity of 91.66% and specificity of 97.91% in this study.

Table 1: Age and Sex Distribution of Diarrhea and Rotavirus positive Cases

Age	Males	Rotavirus Positive Male Children	Females	Rotavirus Positive Female Children	Total No. of Children	Total No. of Rotavirus Positive Children
6 – 12Months	26	5	14	3	40	8
1 – 2 years	24	7	16	5	40	12
2 – 3 years	14	2	8	0	22	2
3 – 4years	6	1	4	0	10	1
4 – 5 years	4	0	4	1	8	1
Total(6m- 5years)	74	15	46	9	120	24

Table 2: Monthly Distribution of Rotavirus Positive Cases

Month	Number of Cases	Number of Rotavirus Positive Cases
June	9	0
July	10	1
August	7	1
September	9	1
October	13	3
November	9	3
December	12	4
January	16	4
February	9	3
March	9	1
April	9	2
May	8	1
Total	120	24

Table 3: Rotavirus Antigen Detection by Different Methods in children with Diarrhea in Various Age Groups

	Total	ELISA	LA	ICG
Age	**No. of cases**	**No.(+/-)**	**No. (+/-)**	**No.(+/-)**
6-12 months	40	8/ 32	9/31	10/30
1-2 year	40	12/28	13/27	11/29
2-3year	22	2/20	3/19	2/20
3-4 year	10	1/9	1/9	1/9
4-5 year	08	1/7	0/8	0/8
Total	120	24/96 (20%)	26/94 (21.6%)	24/96(20%)

DISCUSSION

In the present study rotavirus prevalence was found to be 20% among children of age group six months to five years of age. This is in concordance with other studies from India as well. In India, the prevalence of rotavirus diarrhoea varies from 5-71 per cent, in hospitalized children less than five years of age with acute gastroenteritis.[10]The prevalence of rotavirus depends on several factors, including season of sampling, demographic (e.g. age, sex) and ethnic factors, and the specificity and sensitivity of the diagnostic methods. In a multicentre study, conducted by Giri S. *et al.*, rotavirus positivity was found to be 41.7% in the north India compared to 33.1% in the south.[11] Same study also detected that rotavirus positivity

ranged from 23.5% in Tirupati to 49.4% in Trichy. Another multicenter study across India showed 26.4% positivity in stool samples for rotavirus antigen.[12]

In our study, majority of the cases (83.3%) occurred in children younger than 24 months, which is the susceptible expected target age group. Rotavirus infection can be asymptomatic in children more than two years of age due to development of local intestinal immunity owing to previous infection with rotavirus which confers some protection against subsequent rotavirus infection, with repeated infections becoming less severe or asymptomatic. T. Saluja *et al.*, detected that 82% of rotavirus positive children were less than 2 years of age in their study.[12] Girish k. *et al.*, too revealed that rotavirus detection rates were highest in children aged 6-23 months (41.8%), in their study.[13]

It appeared that infants below 4 months of age were initially protected to some extent against rotavirus diarrhea due to presence of maternal antibodies to rotavirus infection. Children seem to have acquired active immunity by 24 months of age. The children between 6 to 12 months are at greater risks of developing rotavirus diarrhea due to declining levels of maternal antibodies.[14]

In present study male and female children constituted 62.5 % and 37.5%, respectively, of total children suffering from rotavirus diarrhea. Current study shows that male children were affected more than female children in a ratio of 1.7:1. This is in accordance with other studies wherein a preponderance of infection was observed in male children. Junaid *et al.*, found that males excreted rotavirus at a significant higher rate than females ($P<0.05$) in their study.[15]

On the contrary there are studies which have shown no association between male and female children. T. Saluja *et al.*, didn't find any significant difference between rotavirus positive male and female subjects (26.5%among males and 26.1% among females).[12]

Rotavirus diarrhea shows a seasonal variation with a higher incidence of the disease in winter months at a low relative humidity. In present study more cases were seen during cooler months than hotter months. There was a higher prevalence from October to February (17 out of 24). This is consistent with a number of studies carried out in India and other developing and developed countries. Giri S. *et al.*, found a distinct seasonality for rotavirus associated diarrhea, with highest prevalence seen during December–February in their multicentric study.[11]T. Saluja *et al.*, showed that rotavirus related hospitalizations were highest from October through March for all the regions, in their multicentric study.[12]

It has also been observed that temperature influences the stability of human and animal rotaviruses contributing to efficient transmission of the human rotavirus. Moreover the influence of low relative humidity in the home has been suggested as a facilitating factor for the survival of rotaviruses on surfaces.[14]

ELISA has been used in most of the studies for detection of rotavirus antigen. It is used widely in diagnostic laboratories because they provide rapid detection of rotavirus antigen in a relatively short time in comparison to other tests, such as virus isolation, molecular techniques etc. However, ELISA is not ideal when only few samples are required or if test has to be carried out at field level like at a primary health center. In this study simple rapid tests like Latex Agglutination and Immunochromatography, have been compared with ELISA for the detection of rotavirus antigen.

COMPARISON OF LATEX AGGLUTINATION WITH ELISA

In present study with latex agglutination test there were 26 rotavirus positive cases out of 120 children. When compared to standard test i.e. ELISA, Latex agglutination showed a sensitivity of 95.8% and specificity of 96.87%.

Al-Ezzy detected low sensitivity of latex agglutination compared to ELISA (61.11%) and a specificity of 90.91%.[19]

The false positive results in our study could be due to subjectivity of reader since latex is read

by naked eye or testing samples with latex after high speed centrifugation affects results and may be a possible cause of false positive results.[17]Therefore all the new generation latex tests now on the market use a filtration process of the stool samples.[19]
Many studies have been compared LA with ELISA and variable sensitivity and specificity of the LA commercial kits were observed.[18]
Raboni SM *et al.*, have detected 69%, and 100%, as sensitivity and specificity, respectively, of LA when compared with ELISA. Chakravarty *et al.*, too investigated incidence of rotavirus in 145 children with gastroenteritis by LA and ELISA. The sensitivity and specificity of LA was 91.4% and 98.18%, respectively. It showed a high specificity and a reasonable amount of sensitivity and the results correlated well with ELISA.[20]
LA kit is of low complexity, requires less complicated apparatus, is easy to interpret, and provides a rapid diagnosis in a short time. It showed a reasonable amount of sensitivity and a high degree of specificity hence it is a suitable screening test to detect rotavirus in the stools of children with diarrhea.

COMPARISON OF IMMUNOCHROMATOGRAPHY WITH ELISA

In present study, out of 120 patients there were 24 rotavirus positive cases, with Immunochromatography test. When compared to standard test- ELISA, immunochromatography showed a sensitivity of 91.66% and specificity of 97.91% in this study. ICG test requires less handling of the sample, and the results would be available in minimal time.[21]
Momenzadeh A et al., compared Immunochromatography test with ELISA for detection of rotavirus. They found the sensitivity and specificity of ICG to be 87.7%, 98.6%, respectively, which is closely similar to present study.[21] de Rougemont A. showed Sensitivity and specificity of immunochromatographic test and ELISA were comparable; 96.6% and 96.4%, respectively.[22]
Dewar J *et al.,* found out sensitivity of 88% and a specificity of 100% of ICG when compared with the ELISA**.**[23] Sanjoy Kanti Biswas *et al.,* considered ELISA as a gold standard test and found out Sensitivity of ICT to be 80.7% whereas specificity was 100%.[24]
Conclusion: Detection of rotavirus diarrhea among paediatric patients has become very simple and rapid due to development of sensitive immunoassays like Latex agglutination test and immunochromatography test. ELISA has the advantage to be the most sensitive and specific while Latex agglutination test and immunochromatography test have the advantage of being the quicker methods with considerable sensitivity and specificities. They require no specialized equipment and are useful for testing single specimen. A rapid diagnosis of severe rotavirus will prevent unnecessary administration of antibiotics and thus prevent the emergence of antimicrobial resistance in the hospitals.

REFERENCES

1. Pang X and Hodinka RL. In: Gastroenteritis Viruses. Manual of clinical microbiology. Jorgensen JH and Pfaller MA (eds). 11th Ed, Washington, DC: ASM press. 2015: 1617-1632.
2. Shadi TN, Seyed Reza M, Amir G, Seyed MH. Human rotavirus in Iran; molecular epidemiology, genetic diversity and recent updates on vaccine advances. *Spring*. 2019;12(2):98-109.
3. Bass ES, Pappano DA and Humiston SG. Rotavirus. Pediatr Rev.; 2007, 28: 183-91. Doi: 10.1542/pir.28-5-183, PMID: 17473123
4. Bernstein DI, Ward RL. Rotaviruses. In: Feigin RD, Cherry JD, eds. Textbook of Pediatric Infectious Diseases. 5th ed. 2004, Vol 2. Philadelphia, PA: Saunders; 211033
5. Zuccotti G, Meneghin F, Dilillo D, et al. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. BMC Infect Dis. 2010; 10 (218): 1-8.

6. Estes MKKA. Rotaviruses. In: Knipe DM, Howley PM, editors. Fields virology. 5th ed. Lippincott, Williams and Wilkins. 2007:1917-1994.
7. Crawford SE, Ramani S, Tate JE, et al. Rotavirus infection. Nat Rev Dis Primers. 2017; 3:17083.
8. Ferreira T, Becho MC, Bernardo AR, Chaves TCB, Ribeiro RS and de Lima JS. Performance of A Latex Agglutination Test in The Diagnosis of Acute Gastroenteritis by Rotavirus. Brazilian J. Microbiol.; 2006,37:587-9. doi: 10.1590/S1517-83822006000400035
9. Yasmon A, Harahap ED, Dwipoerwantoro PG and Dewi BE. Detection of human group A and C Rotaviruses in pediatric patients with acute gastroenteritis by real time RT-PCR assay: A preliminary study. Makara Kesehatan; 2010, 14(2): 65-9
10. Broor S, Ghosh D, Mathur P. Molecular epidemiology of rotaviruses in India. Indian J Med Res. 2003 Aug; 118:59-67. PMID: 14680200.
11. Giri, S., Nair, N.P., Mathew, A. *et al.* Rotavirus gastroenteritis in Indian children < 5 years hospitalized for diarrhoea, 2012 to 2016. BMC Public Health19**,** 69 (2019).
12. T. Saluja, S.D. Sharma, M. Gupta, R. Kundu, S. Kar, A. Dutta, M. Silveira, J.V. Singh, V.G. Kamath, A. Chaudhary, J.V. Rao, M.D. Ravi, S.R.K. Murthy, S. Babji, R. Prasad, R. Gujjula, R. Rao, M.S. Dhingra. A multicenter prospective hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children less than five years of age in India, Vaccine, Vol 32, Supplement 1, 2014, Pages A13-A19,
13. Girish Kumar CP, Giri S, Chawla-Sarkar M, Gopalkrishna V, Chitambar SD, Ray P, Venkatasubramanian S, Borkakoty B, Roy S, Bhat J, Dwibedi B, Paluru V, Das P, Arora R, Kang G, Mehendale SM; National Rotavirus Surveillance Network investigators. Epidemiology of rotavirus diarrhea among children less than 5 years hospitalized with acute gastroenteritis prior to rotavirus vaccine introduction in India. Vaccine. 2020 Dec 3;38(51):8154-8160.
14. Saravanan P, Ananthan S, Ananthasubramanian M. Rotavirus Infection among infants and young children in Chennai, South India, Ind. J Medical Microbiology 2004; 22(4): 212-221.
15. Junaid SA, Umeh C, Olabode AO, Banda JM. Incidence of rotavirus infection in children with gastroenteritis attending Jos University teaching hospital, Nigeria. Virol J. 2011;8:233.
16. Lorestani et al. Molecular and serologic characterization of rotavirus from children with acute gastroenteritis in northern Iran, Gorgan BMC Gastroenterology (2019) 19:100
17. Ghazi HO, Khan MA, Telmesani AM, Idress B, Mahomed MF. Rotavirus Infection in Infants and Young Children in Makkah, Saudi Arabia. J Pakis Medical Asso 2005; 55: 231.
18. Raboni SM, Nogueira MB, Hakim VM, Torrecilha V TG, Lerner H and Tsuchiya LRV. Comparison of Latex Agglutination with Enzyme Immunoassay for Detection of Rotavirus in Fecal Specimens. Am J Clin Pathol 2002; 117: 392394.
19. Ali Ibrahim Ali Al-Ezzy, SEE J Immunol. 2016 Dec 24; 2016:20008.
20. Chakravarti A, Kumar S, Mittal SK, Broor S. Comparison of latex agglutination and polyacrylamide gel electrophoresis with enzyme linked immunosorbent assay for detecting human rotavirus in stool specimens. Indian Pediatr. 1991 May; 28(5): 507-10.
21. Momenzadeh A, Shahrzad M, Faraji A, Motamedi rad1 M, Sohrabi1 A, Modarres1 S, Azarnoush L, Mirshahabi1 H. Comparison of Enzyme Immunoassay, Immunochromatography, and RNA-Polyacrylamide-Gel Electrophoresis for Diagnosis of Rotavirus Infection in Children with Acute Gastroenteritis. Iran J Med Sci September 2008; 33 (3): 173.
22. de Rougemont A, Kaplon J, Billaud G, Lina B, Pinchinat S, Derrough T, Caulin E, Pothier P, Floret D. Sensitivity and specificity of the VIKIA Rota- Adeno immuno-chromatographic test (bioMérieux) and the ELISA IDEIA Rotavirus kit (Dako) compared to genotyping, Pathol Biol (Paris). 2009 Feb; 57(1): 86-9.
23. Dewar J, de Beer M, Elliott E, Monaisa P, Semenya D, Steele A. Rapid detection of rotaviruses – are laboratories underestimating infection in infants?, South African medical journal July 2005; 95 (7): 494-5.

24. Biswas S K, Sultana N, Md. Badruddoza, Md. Azim A, Roy Biswas R K. Immunochromatographic Test and ELISA for Detection of Rotavirus in Fecal Sample: A Comparative Study, Chattogram Maa-O-Shishu Hosp Med Col Journal Volume 18, Issue 2, July 2019.