



Latex Agglutination Assay- A Rapid Test to Diagnose Rotavirus Infection

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Abstract

Introduction: Diarrhea is one of the most important cause of childhood morbidity. Rotavirus is the commonest cause of viral diarrhea in children. Its symptoms vary from mild to severe diarrhea that results in dehydration. Rotavirus infection can be diagnosed by several methods like culture, electron microscopy, ELISA, latex agglutination etc. Commonly used test in laboratory is ELISA which is more time consuming than latex agglutination test. So we have used latex agglutination kit to detect the prevalence of rotavirus infection in children in Davangere.

Aim and Objective: To detect the presence of rotavirus in stool sample in pediatric age group using latex agglutination test and to know the prevalence of rotavirus infection in children in Davangere.

Materials and Methods: One hundred stool samples were collected from inpatient children aged between six months to five years during the period from June 2010 to June 2011 admitted to Bapuji hospital and Chigateri government hospital, Davangere. Rotavirus antigen was detected by commercial latex agglutination kit, Plasmatec.

Result: Out of 100 samples, rotavirus antigen was detected in 21 samples by latex agglutination test. Total numbers of rotavirus positive male and female children were 13 and 8, respectively, showing a male preponderance. Seventeen children were tested positive for rotavirus antigen during winter (October to February). Maximum cases were seen in the age group of 6 months to 2 years (20) which was statistically significant.

Conclusion: our study showed a prevalence rate of 21%, which is quite high. Latex agglutination test could detect presence of rotavirus in less than one hour compared to ELISA which takes around 3-4hrs. It does not require specialized equipments. Latex agglutination test can be done easily, even in primary health care centers. It helps in quick diagnosis of rotavirus infection which indeed helps in preventing the spread of infection to other children as rotavirus is highly infectious even in low doses.

Keywords: diarrhea, rotavirus, latex agglutination assay, prevalence, ELISA.

Introduction

Gastroenteritis is one of the most important causes of morbidity and mortality in children. Rotavirus is among the common causative organisms causing acute diarrhea in infants and young

children ⁽¹⁾. Approximately one million deaths occur yearly in India due to rotavirus diarrhea. It is also responsible for around 20-30% of childhood hospitalization ⁽²⁾.

Rotavirus was first described in Nebraska calves. In humans, it was first detected by Bishop et al in 1973⁽³⁾. Rotaviruses belong to genus Reoviridae and have double stranded segmented RNA as genome. The mature virus is triple layered and is approximately 70nm in diameter. Rotavirus has six structural viral proteins i.e. VP1, VP2, VP3, VP4, VP6 & VP7 and six nonstructural proteins (NSP1-NSP6). Based on VP6 capsid protein rotavirus is classified into seven different sub-groups (A-G). Out of these groups, only A, B and C are documented to be infective to human beings causing severe cases of pediatric diarrhea⁽⁴⁾.

Rotavirus infection can be mild, moderate or severe. It varies from no symptoms to severe diarrhea, vomiting, fever and finally severe dehydration⁽⁵⁾. In few patients, a rise in liver enzyme level along with pulmonary infiltration is noted. Febrile convulsions have also been noted in rotavirus infection, though it's rare⁽⁶⁾.

Almost all the children less than five years of age will get infected with rotavirus infection at least once, with a peak in cases seen during six months to two years of age. Severe gastroenteritis is seen before one year of age⁽⁵⁾. Infected children shed virus and spread the infection to other children. Patients can excrete virus up to nine days. Incidence of rotavirus diarrheal cases increases during winter months.⁽¹⁾

Rotavirus infections are not usually seen in adults as they have immunity due to childhood infections. Moderate to severe diarrhea due to rotavirus infection occurs in infants and children; it causes dehydration which leads to malnutrition, hence making them vulnerable for other infectious diseases. Therefore, rotavirus infection has a crucial impact on morbidity and mortality of children⁽³⁾. It also causes economic burden to family due to treatment cost and absence from work by parents to attend the sick child⁽⁵⁾.

Various methods are used to diagnose rotavirus infections. These include viral culture, electron microscopic examination, pulsed field agarose gel electrophoresis, ELISA, etc. Culture of rotavirus is cumbersome and cannot be done in routine

microbiology laboratories. Similarly, electron microscopy requires specialized research laboratory. However, commonly used method is ELISA. Since, ELISA is not cost effective for single or few samples; we have used latex agglutination kit to diagnose rotavirus infection.

Materials and Methods

One hundred stool samples were collected from inpatient children aged between six months to five years during the period from June 2010 to June 2011 admitted to Bapuji hospital and Chigateri government hospital, Davangere. Patients having watery, greenish and foul smelling stools were included in the study. Patients with blood stained stools were excluded from the study. Stool samples were collected in a sterile bottle and transported to the microbiology laboratory as soon as possible. The samples were kept at -20 °C until evaluation.

Rotavirus antigen, VP6 antigen, was detected by commercial latex agglutination kit, Plasmatec. Tests were done according to manufacturer's instruction. The brief procedure is as follows- 0.2 ml of fecal specimen was added to a 2 ml aliquot of diluted extraction buffer (1:5), provided in the kit. This gives a 10% suspension. Then this sample was centrifuged for 10 minutes at 2500 rpm. A drop of supernatant from the fecal sample was placed onto the pre-marked circle on the test slide. One drop of the test latex reagent was added next to the drop of fecal sample and mixed with a stirrer. Test slides were tilted backwards and forwards approximately once every two seconds for two minutes. Presence of agglutination indicates a positive test. A negative result was indicated by milky appearance without any visible agglutination of the latex particles.

Positive and negative controls provided in the latex agglutination kit were also put up with the test samples.

Results

Out of 100 samples, rotavirus antigen was detected in 21 samples by latex agglutination test

(Table 1). Total numbers of rotavirus positive male and female children were 13 and 8, respectively, showing a male preponderance (table 3). Rotavirus diarrhea occurred throughout the year with a distinct peak in winter (October to

February), i.e. 17 children were tested positive for rotavirus antigen (graph 1). Maximum cases were seen in the age group of 6 months to 2 years (20) which was statistically significant. (Chi-square test, $\chi^2= 7.86$, p value < 0.05)

Table 1: Total diarrheal cases and rotavirus positive cases.

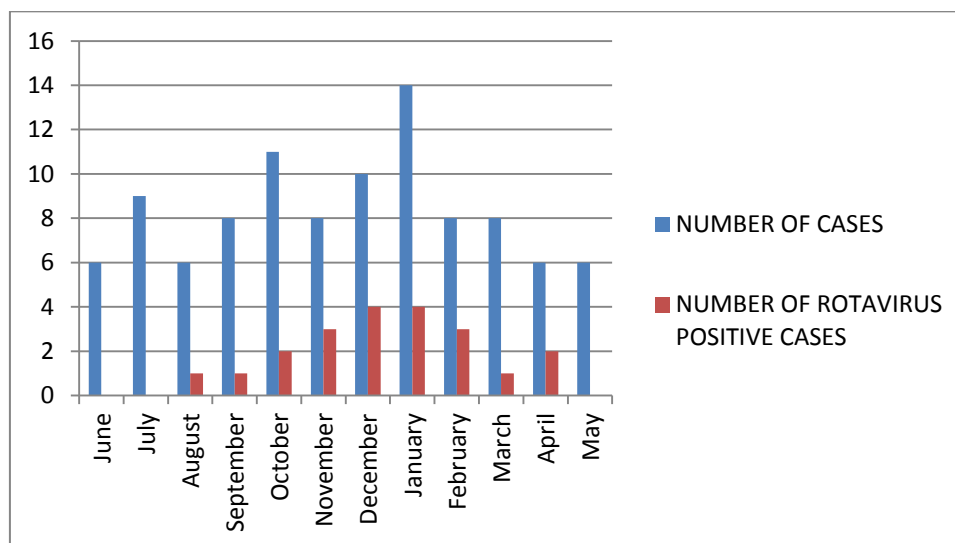
Age	Total No. of diarrhea cases	No. of LA positive cases
6-12 months	34	07
1-2 year	36	11
2-3year	17	2
3-4 year	7	1
4-5 year	06	0
Total	100	21

Table 2: Age and gender Distribution of Diarrhea Cases

Age	Male children with diarrhea	Female children with diarrhea	Total
6 – 12 Months	22	12	34
1 – 2 years	22	14	36
2 – 3 years	11	6	17
3 – 4 years	4	3	7
4 – 5 years	3	3	06
Total (6months-5years)	64	36	100

Table 3: Age and Sex Distribution of Rotavirus Positive Diarrhea Cases latex agglutination.

Age	Male children with rotavirus diarrhea	Female children with rotavirus diarrhea	Total
6-12 Months	5	2	7
1-2 years	6	4	10
2-3 Years	1	0	1
3-4 years	1	1	2
4-5 years	0	1	1
Total (6months-5years)	13	8	21



Graph 1: Month wise distribution of rotavirus infected children

Discussion

The present study was undertaken to discern the prevalence of rotavirus diarrhea using latex agglutination kit. In our study we have detected VP6 antigen of rotavirus. It was detected in 21 patients out of 100, giving a prevalence rate of 21%. This is in association with other studies done in India and around the world. Mathur MS observed that out of 80 test samples, 16 (20%) were positive for rotavirus antigen by latex agglutination kit, similar to our study⁽⁷⁾. Raboni S M et al detected a prevalence rate of 15.4% by LA⁽⁶⁾. P.K. Khatib et al detected a higher prevalence rate (50.4%) of rotavirus diarrhea. The majority of rotavirus-positive cases were observed in children younger than 23 months (62.5%) which is similar to our study⁽⁸⁾. Majority of cases were seen in the age group of 6 months to 2 years. With repeated infections children acquire immunity against rotavirus; hence it is not usually seen in children older than five years and in adults.

Our study also showed a male preponderance as shown by many other studies. Raboni SM et al had also encountered a male preponderance (54.6%) in their study with a male to female ratio of 1.6:1⁽⁶⁾.

There was a seasonal hike in cases of rotavirus diarrhea. More cases were seen in winter than in summer. This has been reported previously by many researchers. Chakravarti A, detected a peak in rotavirus infection in early winter months⁽¹⁰⁾. Bahl R et al observed a distinct peak during winter in children hospitalized due to rotavirus⁽¹¹⁾.

We have used Latex agglutination kit in our study, though ELISA is more commonly used. ELISA needs more specialized equipments like ELISA reader and washer. It is not feasible for few samples and also not very cost effective compared to latex agglutination. On contrary latex agglutination can be done easily on a single test. It does not require special equipments and it is easy to perform in comparatively short time. Latex agglutination tests are apt for field testing as well. Results can be interpreted easily without the use of a mechanized reader.

Latex agglutination kit can be used for screening of gastroenteritis cases. A rapid diagnosis is helpful for better management of diarrhea patients. Since rotavirus is highly contagious, the patient can be isolated to prevent spread of nosocomial gastroenteritis with early and rapid diagnosis. Early diagnosis also helps in rapid rehydration of the patient as rotavirus infection can lead to severe dehydration causing patient death. Latex agglutination will also be of immense help in case of an outbreak of gastroenteritis of rotavirus, as the diagnosis can be made easily and rapidly⁽⁶⁾.

To prevent unnecessary administration of antibiotics and to start appropriate treatment in rotavirus diarrhea patient, a rapid and simple test is required. Latex agglutination test which does not require specially designed equipments is becoming choice of test for the diagnosis of rotavirus diarrhea in microbiology laboratories.

References

1. Tadeusz Chudzio, Shanti Kasatiya, Neil Irvine, Perin Sankar Mistry. Rapid screening test for the diagnosis of rotavirus infection. J Clin Microbiol, Oct 1989: 2394-2396.
2. P Saravanan, S Ananthan, M Anantha-subramanian. Rotavirus Infection Among Infants And Young Children In Chennai, South India. Indian Journal of Medical Microbiology 2004;22 (4):212-221.
3. Younis Abdul-Redha Al- Khafaji, Hawraa J. Al-Jiboury. Detection of rotavirus in diarrhea stool samples of children with acute gastroenteritis in babylon governorate, iraq. Int Research J of Microbial 2013;4(3):84-88.
4. Sasirekha Ramani, Gagandeep Kang. Burden of disease & molecular epidemiology of group A rotavirus infections in India. Indian J Med Res. 2007 May; 125(5):619-632.
5. Meloni A, Locci D, Frau G, Masia G, Nurchi AM, Coppola RC. Epidemiology

- and prevention of rotavirus infection: an underestimated issue? *J Matern Fetal Neonatal Med.* 2011 Oct;24 (Suppl) 2:48-51.
6. Sonia M. Raboni, Meri B. Nogueira, Valeria M. Hakim, Vivian T.G. Torrecilha, Henrique Lerner, Luine R.V. Tsuchiya. Comparison of latex agglutination with enzyme immunoassay for Detection of Rotavirus in Fecal Specimens. *Am J Clin Pathol* 2002;117:392-394
 7. Mathur MS, Bhave GG. Comparative evaluation of coagglutination and latex agglutination test (Rotalex kit) for detection of rota virus. *J Postgrad Med* 1993;39:130.
 8. PK Khatib, ZH Khan. Prevalence of Rotavirus Diarrhoea Among Children in Akola. *Int J of Pharm Res & All Sci* 2013; 2(1):65-69.
 9. Hendra Salim, I Putu Gede Karyana, I Gusti Ngurah Sanjaya-Putra, Soetjiningsih Budiarsa and Yati Soenarto. Risk factors of rotavirus diarrhea in hospitalized children in Sanglah Hospital, Denpasar: a prospective cohort study. *BMC Gastroenterol.* 2014;14:54.
 10. Chakravarti A, Kumar S, Mittal SK, Broor S. Clinical and epidemiological features of acute gastroenteritis caused by human rotavirus subgroups. *J diarrhoeal Dis Res.*1992 Mar; 10(1): 21-4.
 11. Rajiv Bahl, Pratima Ray, Swati Subodh, Prashant Shambharkar, Manju Saxena, Umesh Parashar, Jon Gentsch, Roger Glass MK Bhan. Incidence of Severe Rotavirus Diarrhea in New Delhi, India, and G and P Types of the Infecting Rotavirus Strains. *JID* 2005;192 (Suppl 1) S115.