

Original Research Article

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Utility of Rapid Antigen Detection for Diagnosis of Rota Viral Infection in Children <2 Yrs

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ABSTRACT

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Rotavirus is a major cause of severe gastroenteritis in young children between 6 months to 2 years of age. This study was done with the aim to determine the utility of rapid antigen detection kit for diagnosis of rota viral infection in suspected cases of diarrhoea in children \leq 2yrs. We found that out of the 32 samples, 06 tested positive for the rotaviral antigen by rapid immunochromatography method. 66.7% of the cases were in 13 – 24 months age group. Detection of Rotavirus infection is necessary in determining the clinical severity as well as finding the prevalence and incidence of this infection in the community. The rapid test kit used is easy to use and inexpensive. This can detect the cases of Rotaviral diarrhoea and be helpful in diagnosis as well as in epidemiological studies. It can also prevent the unnecessary use of antibiotics. The rapid antigen detection kit for diagnosis of rota viral infection in suspected cases of diarrhoea in children \leq 2yrs can be a useful tool.

Introduction

Rota virus is a member of the family Reoviridae, the only RNA virus family that has a double stranded RNA. When observed under electron microscope Rotaviruses have a distinct wheel like appearance. Hence they have been named *rota* which in Latin means wheel. The virus has a genome of 11 segments of double-stranded RNA, of molecular weight 2×10^5 to 2.2×10^6 Daltons. This RNA is present in the core which is covered with a triple layered capsid (Shobha Broor, 2003). Seven groups (A-G) of Rotaviruses have been described and only groups A, B and C infect humans. Group A has multiple strains and causes most of the childhood diseases.

Worldwide Rotaviruses are a leading cause of acute gastroenteritis in infants and young children worldwide, infecting nearly all children by the age of 5, often more than once. Each year rotavirus causes approximately 111 million episodes of gastroenteritis, 25 million outpatient visits, and 2 million hospitalizations in children under age 5 worldwide. The incubation period of rotavirus diarrhoea varies from 1-7 days (Parashar *et al.*, 2003; Parashar *et al.*, 2006).

In infants and young children, there is an abrupt onset of severe vomiting and diarrhea with vomiting usually preceding diarrhoea. Stools are usually loose and watery, mucus

may be present but blood is very rare. Mild to moderate dehydration is seen in 80 per cent of cases and severe loss of fluids and electrolytes may be fatal if untreated. Mild fever is seen in a large majority of cases. The illness usually lasts 3-8 days, but virus shedding continues for about 10 days to 1 month. In immunodeficient children, rotavirus can persist for months. Older children and adults are infected but they generally suffer from subclinical infections and virus is infrequently detected in their stool samples (Steele, 1999).

Rotavirus diarrhoea may show a seasonal variation with a high incidence of the disease in winter months at low relative humidity in north India. Treatment of acute rotavirus infection is nonspecific and involves management of symptoms and, most importantly, maintenance of hydration (Bhautik Modi, 2013).

Transmission of Rotavirus occurs through the feco-oral route (Deepali Masurkar, 2013). Rotavirus is continuously shed in large numbers during the course of disease and stool specimens collected from the first to fourth days of illness are optimal for rotavirus detection (Fischer and Gentsch, 2004). They can be easily identified on electron microscopy of stool samples which is one of the most specific tests for diagnosis. Direct electron microscopy examination of stools for rotavirus has a high sensitivity. However it requires expensive equipment and trained personnel, hence cannot be used in field studies. Other methods like immunoelectro osmophoresis and modified complement fixation test were developed, but they lacked sensitivity.

The recent advent of antigen detection methods based on immunological techniques using monoclonal and polyclonal antibodies has gained attention of researchers. Direct detection of viral antigen by rapid one step

immuno-chromatography technique is an inexpensive, easy to handle sensitive test with no need of invasive procedures and special instrumentation (Sushmita Roy *et al.*, 2008).

It is estimated that 1 in every 250 children born in India dies from rotavirus by the age of 5 yr. India accounts for 17 per cent of the world's estimated rotavirus associated deaths. A number of studies have been conducted on the prevalence of childhood rotavirus diarrhoea in various parts of the country in which rotavirus was detected in 5 - 71 % of the hospitalized children less than 5 years of age with acute gastroenteritis.

The clinical manifestations of rotavirus diarrhoea alone are not very distinctive to permit exact diagnosis hence testing of samples in the laboratory is the best way to confirm the diagnosis. Most cases of diarrhoea are treated with antibiotics, irrespective of the causative agent.

However, if infection due to rotavirus can be diagnosed early, the misuse and unnecessary usage of antibiotics can be avoided. Etiological diagnosis may not be essential in the treatment of individual patients, but the knowledge of the relative importance and seasonal prevalence of different pathogens in different regions is essential for proper management of outbreaks and for the planning and implementation of control measures.

In this study, stool samples from cases of diarrhoea patients' ≤ 2 years of age were tested to detect the Rotavirus antigen in the stool specimen by immuno-chromatography test with an aim to know the cause of diarrhoea. If it is of viral origin, unnecessary administration of antibiotics can be prevented and thus help in right and proper management of the patient.

Materials and Methods

This cross-sectional prospective study was conducted in a tertiary care hospital of Bhopal and samples were collected from patients attending the pediatric IPD and OPD for a period of Two months – July 15th to September 15th, 2016.

Cases were defined as children whose main complaint was acute diarrhea, characterized by occurrence of three or more loose, liquid or watery stools with or without mucous in a 24 hours period. Other symptoms like fever, vomiting etc were also recorded.

SD Bioline kit, Lot no. 14BD0034 Expiry date: 2018/06/29 was used for the test. This kit uses rabbit polyclonal anti-rotavirus antibodies which enables identification of Group A Rotavirus antigens.

Stool sample from 32 patients \leq 2yrs of age with diarrhoea were collected in a clean wide mouth screw capped bottle. The samples were transported immediately to Microbiology Laboratory.

They were then processed as per instructions of the manufacturer. Briefly, a portion of faeces (about 50g) from a stool sample was taken and the swab provided was placed into the sample collection tube and swirled at least 10 times. The swab was squeezed against the wall of the tube and discarded. The dropping cap was placed on the sample collection tube. 3-4 drops of the prepared sample was dropped into the immuno-chromatography device, the results were read after 10-20 minutes and appearance of test line was taken to be positive.

Results and Discussion

A total of 32 samples were collected from patients \leq 2yrs of age with diarrhea. Out of

these 12 patients were from the Out-patient department and 20 were admitted to the Paediatric ward. There were 18 male and 14 female patients with distribution as shown in table 1.

Out of the 32 samples, 6 (18.75%) were positive for rotavirus antigen. Out of the 6 positive cases, 1 (16.67%) was a patient from OPD while 5(83.33%) were from the ward. Age wise distribution of the cases were as shown in table 2.

Among the 06 positive cases 03 (50 %) were males and 03 (50 %) were females and one male among them was an OPD patient.

The average duration of the diarrhoea was 7 days and the average frequency was 6 times a day in our study. Fever was present in all the patients with rotavirus diarrhoea with vomiting in 83.3% (5) of the patients. None of them displayed any signs of dehydration.

Acute gastroenteritis remains a leading cause of post-neonatal under-five mortality in India contributing about 13% of under-five mortality. Rotavirus is the most important cause for severe gastroenteritis in this age group. Studies in the last decade estimate the annual mortality due to rotavirus in India to be between 90,000 and 153,000 (Jacob John *et al.*, 2014).

Of India's more than 2.3 million annual deaths among children, about 334 000 are attributable to diarrhoeal diseases. Rotavirus is the leading cause of severe diarrhoea in children in developed and developing countries. Almost all children have been infected by the time they reach five years of age. In developing countries rotavirus is responsible for approximately half a million deaths per year (Bhautik Modi, 2013). The immuno-chromatography test (ICT) used for detection of Rota virus in stool samples give

rapid results. According to Salwa Badrelsabbha Ibrahim *et al.*, (2015) the ICT is quick, inexpensive, easy to perform and requires very little equipment. Jayoung Kim *et al.*, (2014) have reported that rapid tests show no interference, no cross reactivity, high reproducibility and acceptable agreement rates with other detection technologies like ELISA, ELFA and PCR.

In our study the 18.75% of the cases tested were positive for rotaviral antigen by rapid immune-chromatography test. This is in

concordance with studies by Razaq Hadi Eissa *et al.*, (2014) and Jayoung Kim *et al.*, (2014) However other studies have found higher percentages like Sushmita *et al.*, (2012)- 52.5%. Hussein (2013) found that distribution of Rotavirus among infants with diarrhea was 50.5% (52/103). About the distribution of these viruses among age groups, the results show that the most affected age group was 1- 4 months (51.5%) followed by less than 1 month group which consist 34%.

Table.1 Distribution of the patients – gender wise and OPD/IPD

	Male (n=18)	Female (n=14)
OPD (n=12)	6	6
IPD (n=20)	12	8
Total	18	14

Table.2 Age wise distribution of the cases

Age	Positive (n=6)	Negative (n=26)
0-12 months	2 (33.3%)	10 (38.5%)
13-24 months	4 (66.7%)	16 (61.5%)

Among the 6 patients, 01 patient was from OPD while rests 05 were from paediatric ward, suggesting the seriousness of the disease.

In our study, 4 (66.7%) of the positive cases were in the age group of 13-24 months. Similar result has been reported by Salwa Badrelsabbha Ibrahim *et al.*, (2015) with patients in 6-12 months range having highest rate of rotavirus infection - 54%. As far as gender distribution was concerned, there was no significant difference in the distribution of patients or in the number of cases, suggesting that the patients of both genders are equally affected by the disease. In contrast to study by Wg Cdr John *et al.*, (2014) whose study showed 33.2% had fever and 43.6% had vomiting along with diarrhea and study by

Hussein *et al.*, (2013) had 78.6% fever and 68% vomiting symptoms, in our study all the patients presented with fever while 05 patients among the 06 positive cases had vomiting. One patient from OPD did not have vomiting. All the 06 patients were treated symptomatically and all of them recovered without administration of antibiotics.

In a study from Punjab, rotavirus infection has been observed throughout the year with maximum occurrence in November and another peak in the hot and dry months of May (Ram, 1990). The maximum incidence in Pune occurred in winter and the minimum in the rainy season (Kelkar, 1997). This study was conducted during the period of July to September, which can probably explain the low number of positives. Also the number of

samples tested was 32 and a larger number of samples will have to be tested to make the result statistically significant. This was the limitation of our study.

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