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## Research Article

### PREVALENCE OF ROTAVIRUS INFECTION IN CHILDREN BELOW 5 YEARS WITH ACUTE GASTROENTERITIS IN AN URBAN TEACHING HOSPITAL

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#### ABSTRACT

**Background and Aim;** Globally, the deaths due to diarrhea are one among the major leading causes of mortality in children below five years of age. Among the various organisms causing diarrhea, viruses are the most common causative agent. Adenovirus, Rotavirus and Astrovirus are few viruses which cause diarrhea. Almost 66.7% of diarrheal deaths in children below five years of age are caused by Rotavirus. To estimate the prevalence of Rotavirus infection in children below five years of age suffering from acute gastroenteritis.

**Material and methods:** A cross sectional study was conducted for a period of two months for a sample size of 60 at Apollo general hospital, Hyderabad. Rapid ICT test was done for the identification of Rotavirus infection taking diarrheal samples and detecting VP6 antigen present in the middle layer of the capsid of rotavirus which is responsible for acute gastroenteritis in children below five years of age.

**Result:** Out of 60 patients below five years of age suffering from acute gastroenteritis, 11 isolates were positive. By this study we can conclude that most of the acute gastroenteritis cases in children below five years of age are rotavirus infection and without the evidence of causative organism, the administration of anti bacterial agents leads to antibiotic resistance. This study has provided evidence that, majority of the gastroenteritis cases are caused by rotavirus and the need for the modification of treatment regimen is a must.

**Conclusion;** In our study the prevalence of Rotavirus infection in children suffering from acute gastroenteritis below five years of age is 18.3%. The test performed to estimate the prevalence of Rotavirus infection is Immunochromatographic test which is a rapid diagnostic test.

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#### INTRODUCTION

Acute gastroenteritis is an inflammatory condition of the stomach and the small intestine; this can be caused due to many organisms like bacteria, viruses and parasite. Globally, the deaths due to diarrhea are one among the major leading causes of mortality in children below five years of age. Among the various organisms causing diarrhea, viruses are the most common causative agent. Adenovirus, Rotavirus and

Astrovirus are few viruses which cause diarrhea. Almost 66.7% of diarrheal deaths in children below five years of age are caused by Rotavirus. The importance of this project is to show that among the children below five years of age, the most prevalent organism causing gastroenteritis is Rotavirus. The purpose of choosing Rotavirus as my research model is that, acute gastroenteritis is a medical problem in which the child presents with symptoms like acute abdomen, diarrhea without

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passage of blood in stools these symptoms lead to very dangerous complications like dehydration which leads to hypovolemia further leading to circulatory collapse and finally death of the child. There is no specific treatment for rotavirus associated gastroenteritis but the complications can be prevented by giving fluids to the patient and rehydrating the patient. This research would emphasize that the major causative organism causing gastroenteritis is rotavirus.

Globally 13.2% of deaths occurring in children below 5 years are due to diarrhea, [2] of which Rotavirus being the major causative agent [3], [4] and [6]. Rotavirus causes 78,500 deaths, 872,000 hospitalizations and over 3.2 million outpatient visits in children below five years of age all over India [7]. NICED in its report estimated that the total diarrheal deaths in India in 0-6 years of age is 1,58,2009 in the year 2012 [8]. These high cases of gastroenteritis and mortality rates has made it a necessity to conduct a study at a regional level to estimate the endemicity and suggesting a change in the treatment modality. In a study conducted by Anand, *et al.*[10] In the year 1999, most pediatric cases admitted in the hospitals of Hyderabad was diarrhea, out of 352 samples which were tested for rotavirus, 57 samples were positive for rotavirus infection which accounts to 16.2%. The experimental design used in this research is detection of VP6 antigen present in the middle capsid layer. The technique of rapid ICT card test is used, being a rapid detection test for rotavirus; the results were obtained in 15 minutes. The age and name of the patient is noted and stool sample of the patient was collected, by using rapid ICT card test, the stool sample is tested for the presence of antigen, also, history of rotavirus vaccination was asked and noted down, if the guardians of the patient were unaware of the vaccine we informed them about the vaccine and the importance of the vaccine. We also educated the patients about hygienic practices like washing hand before eating since rotavirus spreads by feco-oral route.

With the advent of newer medical techniques and advent of Rotavirus vaccine, the incidents of rotavirus infection have reduced. According to latest study reports, the prevalence of rotavirus infection is about 11.5% to 17.8% [9]. In a study conducted by There are many research works which sated the global burden of rotavirus among children below five years of age, very few research works have been conducted at region level, by knowing the burden of the rotavirus at regional level, we can know about the epidemic of the disease, and precautions can be taken while travelling to that areas, we can also target the case with different approach of treatment. Due to the recent development of rotavirus vaccine, this project can also provide the assessment of the knowledge of rotavirus vaccine. This project also adds on to the need for change in approach of the treatment of diarrheal case and emphasizes to restrict the unnecessary use of antibacterial agents.

Ashok Kumar, *et al.*, (2013) conducted a study on children, in this study various parts of India were tested for the prevalence of rotavirus diarrhoea and the data was compiled. The epidemiology of rotavirus diarrhoea in Hospitalized children and in the community was detected. In this study the prevalence of rotavirus in different regions ranges from 11.5% to 17.8%. Yachha *et al.*,(1988) conducted a study in Chandigarh, where a 218 samples were tested for rotavirus infection of which 25 were positive accounting to 11.5%

prevalence in that area. The peak age incidents were observed in the age group 6 to 11 months. Ramachandran *et al.*, (1993) conducted a study in different areas where 458 samples were tested for rotavirus infection of which 63 samples were positive for the infection accounting to 13.7% prevalence Anand *et al.*, (1998) conducted a study in Hyderabad where 352 samples were collected and tested of which 57 samples were positive for rotavirus infection accounting to 16.2% prevalence in that area. Chakravarthi *et al.*, (1998) conducted a study in Delhi where 560 samples were tested for rotavirus infection, of which 100 samples were positive for the infection accounting to 17.8% prevalence in that area. Nag *et al.*, (2003) conducted a study in Lucknow where 90 samples were tested for rotavirus infection of which 14 were positive for the infection accounting to 15.6% prevalence. The peak incidents were observed in the age group 6 to 12 months.

Sengupta *et al.*, (1979) conducted a study in Manipur on a sample size of 59 of which 53 were positive for rotavirus infection accounting to 89.8% of prevalence in that area in 1979. Shetty *et al.*, (1995) conducted a study in Karnataka on a sample size of 106 of which 19 were positive and 87 were negative accounting to 18% of prevalence in that area. The peak incidents were observed in the age group below 24 months. Samantaray *et al.*, (1980) conducted a study in Delhi on 99 samples of which 32 samples were positive for rotavirus infection accounting to 32.3% of prevalence in that area. The peak incidents were observed in the age group 0 to 6 months. Kelkar *et al.*, (2000) conducted a study in Thane on 39 samples of which 27 were positive for rotavirus infection accounting to 69.2% prevalence in that area. The peak incidents were observed in the age group 6 to 12 months. Banerjee *et al.*, (2002) conducted a study in Vellore on 343 samples of which 94 were positive for rotavirus infection accounting to 27.1% prevalence in that area. Umesh D. Parashar *et al.*,(1998) conducted a study which stated that more than 25% of severe cases of diarrhoea among children below 5 years of age are from developing countries. Global mortality associated with rotavirus among children is high, with more incidents of the disease in countries like India and China, due to poor sanitation and lack of knowledge on rotavirus vaccine. India accounts to 23.2% of the total rotavirus associated deaths. Z Robert F. Raming *et al.*,(2004) conducted a study which stated that the virus attacks the small intestinal villus and cause ischemia due to the release of virus encoded toxin, which stimulates the enteric nervous system and cause damage to the enterocytes. This study also stated that the rotavirus infection is age dependent and affects only children below the age of five as they lack the enzyme protease which makes the virus ineffective. Study aim to estimate the burden of Rotavirus associated gastroenteritis. This study is to support the evidence of unnecessary use of antibiotics, leads to the development antibiotic drug resistance. This study also is an indirect measure of calculating the awareness about the Rotavirus vaccine.

## MATERIALS AND METHODS

The study is conducted by using the Rapid Immunochromatographic test. In this test the VP 6 antigen present on the middle layer of the virus capsule is detected

**Study design:** cross-sectional study

**Site:** Apollo General Hospital, Hyderabad

**Study Population:** Children below five years of age attending Pediatric OPD and IP of Apollo Institute of Medical Sciences and Research, (AIMSR General Hospital), Hyderabad.

**Duration of study:** 2 months

**Sample size:** 60

**Inclusion criteria:** In this cross-sectional study children below 5 years of age attending the pediatric OPD and IP as well as in patients with symptoms of acute gastroenteritis- like nausea and vomiting, diarrhea and abdominal cramps (as per WHO) will be included.

**Exclusion criteria:** Critically ill children and children with persistent or chronic diarrhoea and diarrhoea with mucus and blood and children who have received rotavirus vaccine will be excluded from this study.

**Method:** Rapid ICT card test which detects the VP 6 antigen present in the middle layer of the capsid of the rotavirus. This test is a rapid diagnostic method which detects only a group of rotavirus which infects man. The sensitivity of this test is 100% and specificity is 92.4% [11].

#### **SD Rotavirus BIO LINE**

Samples would be subjected to SD Rotavirus BIO LINE, based on the result obtained, i.e. two bands on the card which reads for test and control indicates that the test is positive, if only one band is present on the card which reads for control, indicates that the test is negative. For each day the total number of the positives and the negatives are noted. At the end the entire data is compiled and analyzed.

**Principle:** A nitrocellulose membrane is pre coated with rabbit monoclonal anti-rotavirus antibodies and mouse monoclonal anti-rotavirus antibodies which are used as detector materials. By using these detector materials, Group A rotavirus antigen can be directly detected from the faecal sample. The faecal sample will specifically react with rabbit anti-rabbit antibodies. The SD BIOLINR Rotavirus test cassette has a letter "T" as test line and "C" as control line on the surface of the device. In the result window initially there are no bands present. The control Line is used for procedural control. When the mixture is added, control line should always appear irrespective of the result obtained which confirms that the reagents of control line are working. A purple "test Line" will be visible in the result window if there are enough rotaviruses in the sample. If rotavirus is not present in the sample, there is no colour appearance in "T".

**Procedure:** The Rapid ICT card test can be divided into two procedures: Preparation of extracted sample: Collect the diarrheal sample in a sterile container.

1. The device and sample are allowed to reach the room temperature.
2. Assay diluent is taken up to the fill line, and then transferred into the given sample collection tube.
3. Repeat the above step.
4. 500 mg of the sample was taken with the sample collection swab.
5. The swab is inserted into the sample collection tube containing assay diluent.

6. Swirl the swab at least 10 times until the sample has been dissolved into the assay diluent and discard the swab while squeezing the swab against the wall of tube.

#### **Test procedure**

1. The test device is taken out from the foil pouch.
2. Dropping cap is assembled on the sample collection tube.
3. 3 -4 drops of the mixture is added into the sample well of the test device.
4. Within 10 to 20 minutes, a purple colour moves across the result window in the centre of the test device
5. The test result is interpreted within 10 to 20 minutes. The test result should not be interpreted after 20 minutes.

#### **Interpretation of the test**

1. A control band will appear on the left section of the result window indicating that the test is working properly.
2. The right side of the result window indicates the test result. If a band (test band) appears on the right side of the window along with the control band it indicates that the test is positive.
3. If only control band is present on the result window, it indicates that the test is negative
4. If control band does not appear on the window, this indicates that the test is invalid.
5. Standard procedure for the collection and disposal of the sample and was followed and all precautions were taken while performing the test. The specimen was tested soon after the collection; in cases of delay they were stored in refrigerators at a temperature of 2 to 8oC. Samples which were stored for more than 72 hrs were not tested. The sample, collection swabs and the collection container were discarded in respective biohazard containers.

## **OBSERVATION AND RESULTS**

The diarrheal samples from children below five years of age suffering from acute gastroenteritis were collected and tested with rapid ICT card test. At the end the entire data was compiled and analyzed. The results were analyzed based on the results obtained from ICT card test. Out of 60 samples which were tested 11 isolates were positive when ICT was performed

**Table 1** Table summarizes total samples, positive and negative rotavirus infection

Total number of samples obtained	Positive for rotavirus infection	Negative for rotavirus infection
60	11	49

Out of 60 samples of acute gastroenteritis obtained from children below five years of age 11 were positive for Rotavirus infection and 49 were negative for rotavirus infection as shown in Table 1. The percentage of positivity is 18.3% shown in figure 1. Rapid ICT card test is a reliable test for the detection of rotavirus in the diarrheal sample and shows 100% sensitivity and 92.4% specificity. It is a rapid detection test for Rotavirus in which VP 6 antigen present in the middle layer of the capsid which is responsible for infection is detected. Table 2 shows the comparison of various studies with the present study. This table also shows that with progression of time the incidents of rotavirus infection has reduced represented in Graph 2, owing to the advancement in health care and the production of Rotavirus vaccine.

### Prevalence of Rotavirus infection

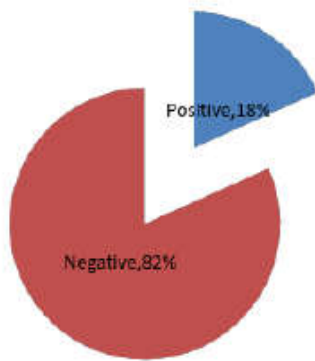


Figure 1 Rotavirus infections in the study population

### Comparison of present study with other studies

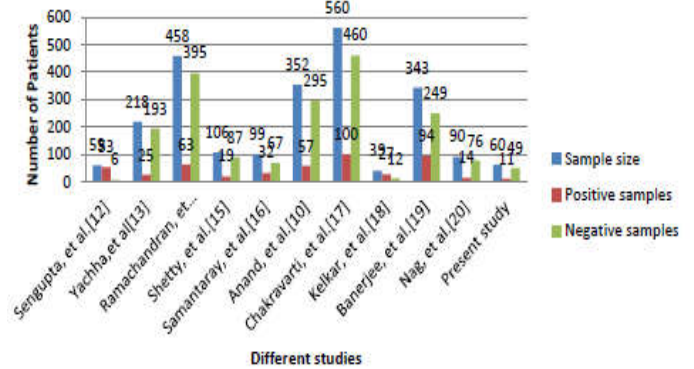


Figure 3 Comparison of present study over the other studies

Graph 2 shows that with the progression of time, the incidents of rotavirus infection has gradually reduced with the peak incidents seen in the year 1979 (89.80%). In a study conducted in the year 2000 -2001 there was a spike in incidents due to an outbreak in Thane, this study was conducted by Kelkar, *et al.*[18]

Table 2 Comparison of different studies with our study

Sl no.	Place of study	Year	Author	Sample size	Positive samples	Negative samples	% of positive samples
1.	Manipur	1979	Sengupta, <i>et al.</i> [12]	59	53	6	89.8%
2.	Chandigarh	1988-1991	Yachha, <i>et al.</i> [13]	218	25	193	11.5%
3.	Multiple	1993	Ramachandran, <i>et al.</i> [14]	458	63	395	13.7%
4.	Karnataka	1995	Shetty, <i>et al.</i> [15]	106	19	87	18%
5.	Delhi	1980-1981	Samantaray, <i>et al.</i> [16]	99	32	67	32.3%
6.	Hyderabad	1998-1999	Anand, <i>et al.</i> [10]	352	57	295	16.2%
7.	Delhi	1998-2000	Chakravarti, <i>et al.</i> [17]	560	100	460	17.8%
8.	Thane	2000-2001	Kelkar, <i>et al.</i> [18]	39	27	12	69.2%
9.	Vellore	2002-2004	Banerjee, <i>et al.</i> [19]	343	94	249	27.1%
10.	Lucknow	2003-2004	Nag, <i>et al.</i> [20]	90	14	76	15.6%
11.	Hyderabad	2016	Present study	60	11	49	18.3%

### Variation of prevalence with progression of time

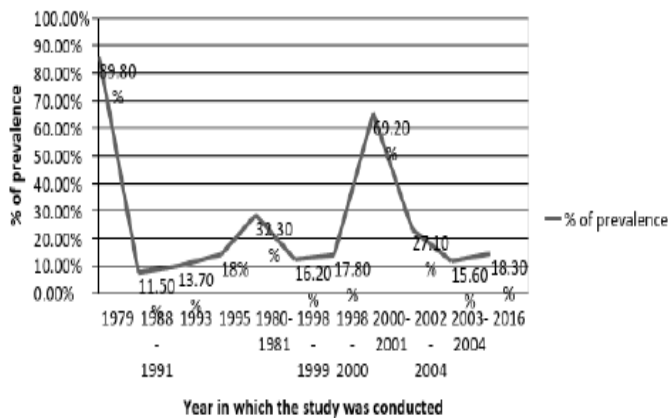


Figure 2 Prevalence of Rotavirus infection in population over the time

In our study out of 60 samples collected 11 were positive, and our study result is in close correlation with studies conducted by Chakravarti, *et al.* (17.8%), Nag, *et al.* (15.6%), Anand, *et al.* (16.2%) study results as shown in Graph 3. We can also observe that with the advent of advanced medical care and production of Rotavirus vaccine the incidents of rotavirus infection are reduced.

### DISCUSSION

Immunochromatographic assay is an antigen antibody agglutination technique that allows the identification of group specific proteins, including the major inner capsid protein, present in group A of rotavirus. The test is based on the detection of VP 6 antigen present on the middle layer of the capsid, ICT test is a rapid test and the specificity of the test is 97%. The antigen VP6 which is responsible for the pathogenesis and causes diarrhea in children is detected by ICT. The nitrocellulose based membrane pre-coated with rabbit monoclonal antibodies and the specially-selected monoclonal anti rotavirus antibodies are used as detector materials and used as detectors. These enable the test to identify Group A rotavirus antigen directly from the sample in human faecal specimens and then this mixture will react specifically with the rabbit anti rotavirus antibody on the membrane. The test is a rapid qualitative test for the detection for the detection of group A rotavirus in human faecal specimen. The ICT test was performed using the standard protocol and the all the rules of WHO were followed. Out of the 60 samples we obtained from children suffering from acute gastroenteritis, 11 were positive. Though humans of all age groups are susceptible to rotavirus infection, the infection shows its severity in children below 5 years of age. The individual suffering from rotavirus infection excretes large amount of virus (rotavirus) which can be spread even through contaminated hands and formites also. The ICT kit used in this study is an immunoassay to detect Group A rotaviruses in the faecal specimen. The major site where the pathogenesis of rotavirus occurs is the intestines, where it causes electrolyte imbalance and decreases the absorption of water from the gut. Rotavirus majorly affects children below five years of age as the lack the enzyme protease which can destroy the VP6 antigen present in the middle layer of the virus capsid and cause pathogenesis. This enzyme appears in the gut only after five years of age, and thus the prevalence of rotavirus infection above five years of age is almost negligible. Our

study on the prevalence of rotavirus in children below five years which gave 18.3% positivity is showing good agreement with other studies conducted by Chakravarti, *et al.* (17.8%), Nag, *et al.* (15.6%), Anand, *et al.* (16.2%).

## CONCLUSION

In our study the prevalence of Rotavirus infection in children suffering from acute gastroenteritis below five years of age is 18.3%. The test performed to estimate the prevalence of Rotavirus infection is Immunochromatographic test which is a rapid diagnostic test. ICT test is a reliable test for the detection of the presence of rotavirus in the children suffering from acute gastroenteritis, being a rapid test and sensitivity of the test being more than 97%, the results can be obtained within 15 to 20 minutes, this saves the time and helps in deciding the treatment regime and avoids the unnecessary prescription of antibiotics. The approach of treatment of a diarrhea case in children below five years should be modified. By seeing the prevalence percentage of rotavirus we can say that the disease caused in children below five years of age is predominantly rotavirus infection and administration of anti bacterial agents without a laboratory diagnosis is futile and contributes to antibiotic resistance.

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