

Original Research Article

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Study on Prevalence of Diarrhea among Children with Special Reference to Rotavirus in Thanjavur, Tamilnadu, India

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ABSTRACT

Diarrhoeal diseases are the second most common cause for mortality in children below 5 years of age. Life threatening gastroenteritis in children are most commonly caused by Rotavirus infection. Worldwide, more than half a million children under the age of five years die each year due to diarrhea. In 2013, an estimated 215,000 of these deaths were due to Rotavirus, with more than 90% occurring in developing countries. Rotavirus is the cause of 23-39% of diarrhoea in children. A rotavirus infection usually starts with a fever and vomiting, followed by 3-8 days of watery diarrhea. The infection can cause abdominal pain as well. Prevalence of rotavirus infection varies according to age, season and geographical area. The aim of this study is to determine the prevalence of rotavirus and to provide useful epidemiological and clinical data for rotavirus surveillance. About 80 children who are all diagnosed as having acute gastro enteritis and admitted in the pediatric ward were included in this study. This study was done over a period of one year from July 2014 to June 2015. In this study youngest child was one month old and oldest child was 4 years 10 months old. More than 80% of RVGE cases were identified to be under two years of age in Thanjavur district and also it is most prevalent in the winter months with common symptoms of diarrhoea and vomiting. This was the First study using Rota antigen ELISA test for Serological diagnosis of rotavirus in Thanjavur medical college and hospital for 80 samples.

Keywords

Diarrhoea,
Rotavirus,
Gastroenteritis,
Thanjavur

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Introduction

Gastroenteritis is a common illness which can be particularly serious in young children. No specific antiviral treatment is available and antibiotics are not effective. Life threatening gastroenteritis in children are most commonly caused by Rotavirus infection. A Rotavirus infection usually starts with a fever and vomiting, followed by three to eight days of

watery diarrhea. The infection can cause abdominal pain as well. Diarrhoea is major symptom of Rotavirus infection. Villi of the small intestine are infected by Rotaviruses. They multiply in the cytoplasm of enterocytes and damage their transport mechanisms. The mechanism of pathogenesis behind diarrhoea were (a) Reduction in total small intestinal surface area due to cell lysis, leading to a decrease in net fluid absorption (primary

malabsorption) and lead to villous atrophy, (b) changes in the osmotic permeability of the mucosa secondary to cellular destruction, transepithelial resistance is decreased after Rotavirus infection result in increased epithelial permeability (Obert *et al.*, 2000). In young children Intestinal permeability has also been investigated using polyethylene glycols (PEGs). In acute phase of virus infection PEG absorption was significantly lower than during 3 to 5 weeks after infection, (Stintzing *et al.*, 1986) (c) Increased electrolyte secretion due to reactive crypt cell hyperplasia (Salim *et al.*, 1995). Diarrhoea caused by Rotaviruses may be due to impaired sodium and glucose absorption as damaged cells on villi are replaced by nonabsorbent immature crypt cells. Another theory was enzyme lactase which is normally present in the lysozymes and in the brush border of intestinal villi. This enzyme in upper small intestine hydrolyzes the Lactose. When Rotavirus infects these cells which get damaged leads transient lactase deficiency. This pathogenesis cause increase in osmotic activity, increase in intestinal fluid, and causes bloating, distension, cramps, and finally watery diarrhoea. As the infected individuals had secondary lactose intolerance, sugar can be demonstrated in the stools (Ashwin Borade, 2010). The major determinant of RNA segment responsible for pathogenesis is VP4. The other genes responsible for pathogenesis are VP3, VP7, NSP1, NSP2, and NSP4. NSP4 acts as enterotoxin considered as one of the important causes for pathogenesis of Rotavirus infection (Ball *et al.*, 1996). Signal transduction pathway is triggered which induces secretion. Into the lumen of the intestine damaged cells form slough and release large quantities of virus, which appear in the stool (up to 10¹² particles per gram of faeces). Viral excretion usually lasts from 2 to 12 days. Intestine restores its normal function only after 3 to 8 weeks.

Rota virus replication occurs exclusively in cytoplasm of mature epithelial cell (i.e.) differentiated enterocytes at the tips of villi of the small intestine. In vitro replication was studied in secondary monkey kidney cells and in immortalized monkey kidney cell lines (MA104, BS-C1) were there was similar replication as in humans (Estes and Cohen, 1989).

Rotaviruses are very contagious. They are generally transmitted by fecal-oral route. Also transmitted by respiratory route but it is not usual mode of spread. Duration of spread of virus from the stool is from 4–25 days of infection. Transmission is very efficient as these viruses are resistant to physical inactivation. The rapid spread of the infection is due to its ability to survive on various surfaces under different conditions. Recently there are speculations about role of animals as source of Rotavirus infection in humans. Human - bovine reassortant strains, human - porcine reassortant strains, human-simian reassortant strains have been endemic in India (Estes *et al.*, 2013). Rotavirus plays a role in causing Nosocomial diarrhoea in children less than 5 years, it constitutes about 18% of Nosocomial diarrhea (Alrifai *et al.*, 2009). Major risk factor in the incidence of AGE was considered to be Poor sanitation.

The most effective method to prevent and to reduce Rotaviral disease burden is the Vaccination. World health organization's strategic advisory group of experts (SAGE) strongly recommends including rotavirus vaccine in national immunization programme, where under 5 mortality is more than 10%.

Materials and Methods

This study includes of 80 children aged less than 5 years suffering from acute gastroenteritis, admitted in pediatric ward, at tertiary care hospital Thanjavur Medical

college Hospital, Thanjavur during July 2014 to June 2015. All the 80 samples initially tested to rule out bacterial and parasite causes.

Specimen collection

About 10 -15 ml of freshly passed stool samples is collected in sterile, wide mouth, leak proof plastic container from each child under study. Stool samples should be collected within 7 days of onset of symptoms. After the collection, the stool samples were immediately transported to the laboratory for further processing. Collected specimens are stored in deep freezer at -20° C until tested for Rotavirus antigen.

Specimen processing

The stool samples were immediately transported to the laboratory for further processing. Initially faecal samples are subjected to Macroscopic examination and microscopic examination for Colour, Consistency and Atypical components like mucus, blood and parasites.

Then the samples are subjected to microscopic examination by saline and iodine wet mount for RBCs, Leucocytes, and Parasitic ova and cysts.

Saline wet mount

It is prepared by mixing the stool with physiological saline. It is colorless preparation which highlights the staining property of egg, detects motility of trophozoites and other structures like pus cells, RBCs.

Iodine wet mount

It is prepared by mixing the stool with iodine. It is brown colored preparation which highlights the presence of nuclei and

glycogen mass in cyst. The faecal specimens were subjected to culture in order to look for common enteropathogens. The stool sample was inoculated in MacConkey plates and incubated for 18 to 24 hours at 37° C. At the end of the incubation period, the plates were examined for the isolates.

Bacterial isolates were identified by adopting the procedures of Gram staining, motility and routine biochemical reactions like Catalase test, oxidase test, motility test was done followed by Indole test, Methyl Red Test, Voges Proskauer Test, Citrate test, Urease test, nitrate reduction test, TSI test were done and results were recorded. Based on these tests, the organisms were identified. Quality control was also performed for all the standard tests.

ELISA

By using Premier™ Rotaclone ELISA Kit Rotavirus antigen was detected. Rota virus antigen detection was done by ELISA method. A (urea peroxide) and substrate B Tetra Methyl Benzidine (TMB) are added to the wells and incubated for 10 minutes at room temperature. The enzyme bound in the wells converts the colour-less substrate to blue colour. The intensity of the blue colour is directly proportional to the concentration of rotavirus antigen in the sample. Addition of stop solution converted the colour from blue to yellow. Wells were read spectrophotometrically using a 450nm filter.

Results and Discussion

In this study the prevalence of rotavirus was 29%, Table 1 and 2. This value almost rationale with the prevalence rates of rotaviral infections in developing countries. This prevalence rate is similar to prevalence rate (28.57%) of the study by Shetty *et al.*, (2014) at Karnataka.**Error! Bookmark not defined.**

Manohar *et al.*, 2015, states that 25.66% of the children aged under 5 years of diarrhea are due to rotavirus. In India high prevalence were reported from Kerala (70%) and Manipur (89%) due to epidemiological nature of these places.

Out of 80 children in this study, majority of children belongs to 7-12 months of age (36%). In **Error! Reference source not found.** 3 out of 23 (29%) positive children for rotavirus antigen, 20 (35%) were in the age group below 2 years and 3 (13%) were in the age group above 2 years. There was a significant difference between the age group of less than 2 years and age group of more than 2 years ($p < 0.05$). Under five age distribution in positive children in this study is similar to other studies in the world wide by Kurugol *et al.*, 2003; Chavan *et al.*, 2013 and Shetty *et al.*, 2014. According to WHO Scientific working group 6-24 months of age is vulnerable group for RV infections,

In the present study out of 80 children 46 (58%) were male children and 34 (42%) were female children. Out of 23 (29%) positive children, 12 (26%) were male children 11 (32%) were female children. Sex distribution in Rota virus positive and negative was almost similar. With reference to Table 2, there was no significant difference between the sexes in this study ($p > 0.05$). Zafer

Kurugol *et al.*, (2003) stated that prevalence in both male and female children was same. **Error! Bookmark not defined.**

Seasonal distribution in all 80 cases as well as positive and negative cases in this study clearly states that rotavirus infection occurs throughout the year but peak infection occurs during winter months November to January. Out of 30 cases 10 were positive during October to December about 33% of prevalence during these months. Out of 12 cases 5 cases were positive during Jan – March shows prevalence of 42%. Totally there was high prevalence rate during winter months from October to March. There was significant difference of rotavirus prevalence between winter months and other seasons.

The clinical presentation in all 80 cases and Rota viral positive cases were showed in **Error! Reference source not found.** 4. Diarrhoea present in all cases in this study showed 100% prevalence. 87% of RV positive children presented with vomiting which was considered as next predominant symptom. So, prevalence of vomiting was 41%. Percentage of RV positive children presented with fever was about 74%. prevalence rate for fever was 31%. Decreased urine output was present in 13% of RV positive cases, but prevalence rate was about 100% (Table 5).

Table.1 Rotavirus antigen test by ELISA

Result	Total	%
Positive	23	29%
Negative	57	71%
Total	80	100%

Table.2 Gender distribution

Cases	Males	Females
No of cases	46	34
No of RV positive cases	12	11

% of RV positive cases	26%	32%
% of total no. of positive cases	15%	14%

Table.3 Age distribution

Group	Age in months	No. of cases	No. of RV positive cases	% of RV positive cases for particular Age group
Group 1	Less Than 2 years	57	20	35%
Group 2	Older Than 2 years	23	3	13%
	Total	80	23	29%

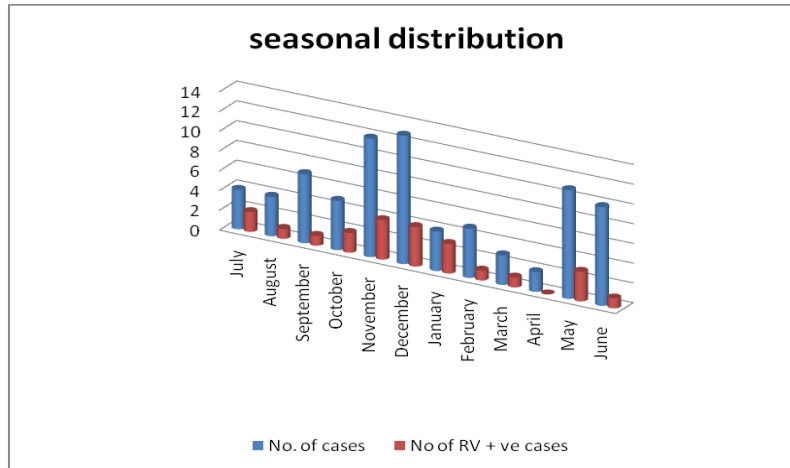
Table.4 Prevalence of clinical symptoms

Symptoms	No. of Cases	No. of RV positive cases	% RV positive Cases
Diarrhea	80	23	29%
Vomiting	51	19	37%
Fever	53	19	36%
↓ Urine Output	3	3	100%
Abdominal pain	4	2	50%
Septicemia	2	0	0%
Shock	1	0	0%
Respiratory infections	2	0	0%

Table.5 Prevalence of bacterial infection

	No. Bacterial isolates
RV positive	2
RV negative	12
Total	14

Chart.1 Seasonal distribution



Abdominal pains were present in 8% of RV positive cases, but the prevalence rate was about 50%. Chavan *et al.*, 2013 and Shetty *et al.*, 2014 stated that diarrhea and vomiting were predominant symptoms in RVGE. This study highlights the prevalence of Rota Viral Gastro Enteritis (RVGE) in under five year children which is 29%. ELISA for detection of rotaviral antigen is the very usual method for early diagnosis of RVGE. This is the First study using Rota antigen ELISA test for Serological diagnosis of rotavirus in Thanjavur medical college and hospital for 80 samples. This study was done to determine the etiological factors for acute diarrhoea in children less than five years in tertiary hospital, Thanjavur. More than 80% of RVGE cases were under two years of age. Prevalence of Rotavirus diarrhoea in this study was about 29%. Rotavirus diarrhoea was more common in the age group of below 2 years. There was no significant difference between the sexes in this study. Rotavirus diarrhoea was common during winter months from October to March. 87% of RV positive children presented with vomiting which was considered as second predominant symptom next to diarrhoea. 58% of the cases reported with severe dehydration in this study. Duration of hospitalization in Rotavirus positive cases was more when compared to negative cases which imply severity of rotaviral infections. Out of 80 samples, 29% were positive by ELISA. Rapid diagnostic procedures like Rota antigen

detection by ELISA provides usual information to the physician, may prevent inappropriate treatment and nosocomial infections by intervening the spread of the infections in hospital set up.

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