

Detection of Rotavirus by ELISA in Stool Samples of Hospitalised Children

Neeraj Kumar¹, Bharti Malhotra², M. Anjaneya Swamy³, S K Mehra⁴

^{1,3}S M S Medical College Jaipur Rajasthan

²Department of Microbiology & Immunology, Advanced Basic Sciences & Clinical Research Laboratory, (ICMR Grade – I Viral Diagnostics & Research Laboratory), Sawai Man Singh Medical College, Jawahar Lal Nehru Marg, Jaipur 302 004, India

⁴Geetanjali University Udaipur Rajasthan

Abstract: ***Objective:** Acute viral gastroenteritis (AGE) is one of the most common disease in children, and affecting all age groups worldwide, causing approximately 2.3 million deaths annually. To study the detection of Rotavirus in Stool samples and their trends in children ≤5 years of age hospitalised at tertiary care centre, Jaipur Rajasthan. **Methods:** Hospitalised children less than 5 years admitted for acute diarrhoea were examined and clinical profile of diarrhoea in the children. Detection of Rotaviral antigen by a commercial Enzyme Immuno Solvent Assay Premier Rotaclone kit (Meridian Bioscience, Inc). **Results:** Out of 144 children, 56 (38.89%) were positive for Rotavirus. The prevalence of Rotaviral diarrhoea in children was found in the age 1-12 months (44, 78.57%) followed by 13-24 months (9, 16.07%), 25-36 months (2, 3.57%), 49-60 months (1, 1.78%) and no virus detected in 37-48 months age groups. Rotaviral diarrhoea was more common during winter and spring months (November-2011 to March-2012), with highest prevalence in months of November-2011 (54.55%), followed by December-2011 (46.15%), January-2012 (47.06%), February-2012 (47.73%) and March-2012 (22.45%). A total of 56/144 (38.89%) samples were found to be positive for rotavirus and among these, 40 (27.78%) were males and 16 (11.11%) were females. **Conclusion:** Rotavirus was detected in 38.89% of hospitalised children with gastroenteritis. This study has highlights that rotavirus diarrhoea accounts for a more percentage of diarrhoeal disease in hospitalised children ≤5 years in Jaipur, Rajasthan and Rotavirus is more prevalent during winter and spring months of the year.*

Keywords: AGE, Diarrhoea, Rotavirus and ELISA

1. Introduction

Acute gastroenteritis is a common infection of young children and adults which is characterized by rapid dehydration of body and is a major cause of mortality in developing countries [1, 2]. There are many reasons for gastroenteritis; viral gastroenteritis is the leading factor among children. It is infection of gastrointestinal tract characterized by inflammation of the inner lining of the stomach, small intestine, and large intestine. The main symptoms of viral gastroenteritis include watery diarrhoea, fever and vomiting. More than one billion episodes of diarrhoea occur every year among children under 5 years of age causing approximately 2.5 million deaths worldwide. The World health organizing (WHO) child health epidemiology reports estimates that 16% of these deaths are in African region [3]. In India diarrhoea is the third most killer disease of children ≤ 5 years of age and kills an estimated 300000 children in India each year [4].

Few patients may show some nonspecific symptoms such as headache, chills and abdominal pain. Symptoms usually show within 12 to 48 hours after contact to a gastroenteritis causing virus infection and last for 1 to 3 days. In India rotavirus is responsible for 40% of children diarrhoea hospitalisation and approximately 527,000 (475,000 - 580,000) deaths annually [5] or 29 to 45% of all death due to diarrhoea among children ≤ 5 year of age globally [6, 7]. Rotavirus is spread independent of age, sex and socioeconomic of the individuals, however young children remain to be the group at risk.

Rotavirus causes approximately 39% of childhood diarrhoea hospitalisation and approximately 1.13 lac deaths annually in children < 5 years of age [8]. The present study was designed to study the prevalence of rotavirus infection in children and to get a better understanding of the seasonal infections and their better treatment measures in the region.

The current study was undertaken to determine the prevalence of rotavirus and clinical profile of diarrhoea among admitted children ≤5 years in JK Lone and attached hospitals in Jaipur.

2. Material And Methods

Patient Inclusion criteria:

Stool samples collected during the period October2011-March2012 who attended the inpatient wards of the Department of General Medicine at J K Lone and SMS Hospitals, Jaipur were enrolled. All children were of ≤5 years of age, presenting with diarrhoea, vomiting, headache, signs and symptoms fever, chills and abdominal pain.

Sample collection and transportation:

A total 144 of stool samples were collected from suspected or clinically diagnosed cases of gastrointestinal tract infections in to a sterile transport container. The stool samples were properly labeled & transported in cold chain (4-8°C) at the earliest to the laboratory and stored the samples -80°C till further processing. The study was approved by institutional ethics committee.

Sample Size: Sample size has been calculated using this formula

Volume 6 Issue 4, April 2017

www.ijsr.net

Licensed Under Creative Commons Attribution CC BY

$N=4PQ/L^2$ (Where N= Total samples, 4= is the factor to achieve the confidence level of 95%, P= known Prevalence that is 9% Q= 100-P and L= permissible (absolute) error, set at 5%)

$N=4 \times 9 \times (100-9) / 5 \times 5 = 131.04$

Sample processing

Macroscopic examination of stool samples was conducted as follows.

- Colour
- Consistency (Solid, Semisolid and Liquid)
- Presence of mucus
- Presence of blood
- Presence of segments and worms

Detection of rotavirus by ELISA

ELISA for RV was performed using Commercial kit, Premier Rotaclone EIA kit (Meridian Bioscience, Inc.) as per manufacturer's instructions. Briefly, the microtitre plates were coated with Rota viral VP6 monoclonal antibody. Presence of viral antigen (VP6) for RV was tested by in a solid phase sandwich ELISA. The antigen is sandwiched between anti RV monoclonal antibody and enzyme linked antibodies.

3. Results

Age and Months wise distribution of children

All the samples were collected from hospitalised children and the subjects enrolled in the study were between 1-60 months of age. The distribution of the children as per age and months was as follows; maximum numbers of children were in the age group of 1-12 months (113, 78.47%) followed by 13-24 months (25, 17.36%), 25-36 months (3, 2.08%), 37-48 months (2, 1.39%) and 49-60 months (1, 0.69%). (Table no.1)

Table 1: Age and month's wise distribution of samples enrolled in study from October 2011- March 2012

Month/Year	1-12 months	13-24 months	25-36 months	37-48 months	49-60 months	Total Samples
Oct 2011	5	5	-	-	-	10
Nov 2011	10	-	1	-	-	11
Dec 2011	13	-	-	-	-	13
Jan 2012	14	3	-	-	-	17
Feb 2012	33	9	1	-	1	44
Mar 2012	38	8	1	2	-	49
Total	113	25	3	2	1	144

Rotavirus among different age groups

Out of 144 children tested, 56 (38.89%) were positive for Rotavirus. The prevalence of Rotaviral diarrhoea in children was found in the age 1-12 months (44, 78.57%) followed by 13-24 months (9, 16.07%), 25-36 months (2, 3.57%), 49-60 months (1, 1.78%) and no virus detected in 37-48 months age groups. In our study higher positivity of Rotavirus was found be in the age groups 1-12 months. (Table no.2)

Table 2: Age-wise distribution of Human Rotavirus in children ≤ 5 year

Age in months	Rotavirus status of children		Total
	Positive	Negative	
1-12	44	69	113
13-24	9	16	25
25-36	2	1	3
37-48	-	2	2
49-60	1	-	1
Total	56	88	144

Distribution of Rotavirus males and females

A total of 144 stool samples hospitalised children of age ≤ 5 years were tested for human rotavirus infection by ELISA, out of which 103 (71.53%) were from males and 41 (28.47%) were from females. A total of 56/144 (38.89%) samples were found to be positive for rotavirus and among these, 40 (27.78%) were males and 16 (11.11%) were females. (Table no.3)

Table 3: Sex wise distribution of Rotavirus in children

	No. of Samples	Negative	Positive
Male	103	63	40
Female	41	25	16
Total	144	88	56

Month-wise distribution of Rotavirus

Rotaviral diarrhoea was more common during winter and spring months (November-2011 to March-2012), with highest prevalence in months of November-2011 (54.55%), followed by December-2011 (46.15%), January-2012 (47.06%), February-2012 (47.73%) and March-2012 (22.45%).

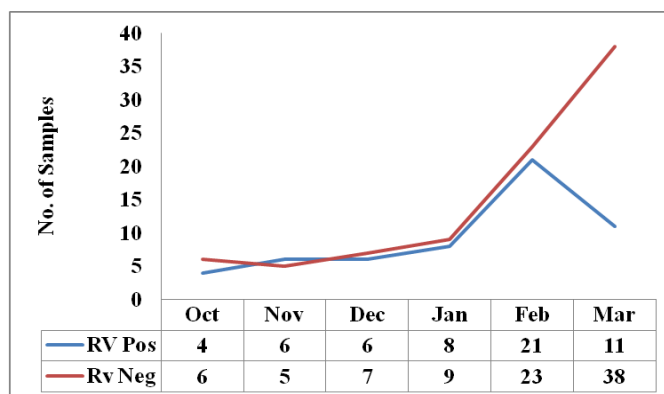


Figure 1: Distribution of Rotavirus from October 2011- March 2012

Association of Rotavirus with different signs and symptoms-

Out of the 56 Rotavirus positive children presented with diarrhoea in 56/56 (100%), fever in (34, 60.71%) children and vomiting was present (40, 71.42%) children and among the 88 Rotavirus negative children, vomiting was present in (64, 72.72%) children and fever was present in (26, 29.55%) children. (Table no.4)

Out of 56 rotavirus positive children, (29, 51.78%) had mild dehydration, (21, 37.50%) had moderate dehydration and (6, 10.72%) had severe dehydration.

Table 4: Positivity of Rotavirus in relation to different signs and symptoms

Symptoms	No. of positive samples	Positivity (%)
Diarrhoea	56	100
Fever	34	60.71
Vomiting	40	71.42
Dehydration	Mild	29
	Moderate	21
	Severe	6

4. Discussion

The present study conducted at a tertiary care centre in Jaipur to study the prevalence and seasonal distribution of Rotavirus diarrhoea hospitalised children aged ≤ 5 years from October 2011-March 2012 for a period of 6 months. Rotavirus is an important pathogen causing diarrhoea and vomiting under 5 years of age children. In India Diarrhoeal disease is a major public health problem among children under 5 years of age. In our study Rotavirus was positive in 38.89% of diarrhoea in hospitalised children, which correlates very fine with other studies conducted in India. Ramani and Kang [9] reported 20-35% positivity and Banerjee [10] reported 23.5% positivity. Rotavirus was the predominant virus found in 49.5% cases in a study done by Swapin [11]. Another study from America reported a prevalence rate of 46.7% for RV infection [12].

RV infection was found to be predominant in hospitalised children ≤ 12 months of age (78.57% cases) in our study. This is in consistence to an earlier study by [13] which reported RV to be an important cause of hospitalisation in children younger than 1 year of age. The RV positivity was found to be 14.28% in less than 6 months age group, 25.4% in 6-10 months, 24.7% between 11 and 15 months and 23.9% between 16 and 20 months in studies done by [10, 14].

In the present study, the overall positivity of Rotavirus was higher in males as compared to females. This is in consistence with an earlier study which revealed higher proportion of infection in male children over females [15]. Study by Nguyen [12] also reported males were more admitted to the hospital due to diarrhoea caused by rotavirus than females ($P=0.06$) and higher prevalence of AGE viruses in males than in females was 1.9 (181 males and 93 females).

In our study, Rotavirus infection was more common during winter and spring months (November to March), with highest prevalence in months of November (54.55%), December (46.15%), January (47.06%), February (47.73%) and March (22.45%). Bahl [16], Nath [17] and Phukan [18] reported similar observation as in this study with peaks during winter months.

5. Conclusion

This study has highlights that rotavirus diarrhoea accounts for a more percentage of diarrhoeal disease in hospitalised children ≤ 5 years in Jaipur, Rajasthan and Rotavirus is more prevalent during winter and spring months of the year.

References

- [1] Logan C, John J, O Leary and Niamh O Sullivan. 2006. Rotavirus and Adenovirus as causative agents of acute viral gastroenteritis in children our Lady's Hospital for Sick *J Clin Microbiol Infect Dis.* **44:** 3189-3195.
- [2] Parkin PC, Macarthur, Khambalia A, Goldman RD and Friedman JN. 2009. Clinical and laboratory assessment of dehydration severity in children with acute gastroenteritis. *Clin. Pediatr.* **49:** 235-239.
- [3] Bryce J, Boschi-Pinto C, Shibuya K and Black RE Lancet. 2005. WHO estimates of the causes of death children. *Lancet.* **365:** 1147-1152.
- [4] Bassani DG, Kumar R, Awasthi S, Morris SK and Paul VK. 2010. Causes of neonatal and child mortality in India Million Death Study Collaborators: a nationally representative mortality survey. *Lancet.* **376:** 1853-1860.
- [5] Charles MD, Holman RC, Curns AT, Parashar UD, Glass RI and Breese JS. 2006. Hospitalizations associated with *Rotavirus* gastroenteritis in the United States 1993-2002. *Pediat Infect Dis.* **25:** 489-493.
- [6] Parashar UD, Glass RI, Jain V and Bhan MK. 2001. Epidemiology of rotavirus in India. *Indian J Pediatr.* **68:** 855-862.
- [7] Parashar UD, Bresee JS and Glass RI. 2003. The global burden of diarrhoeal disease in children. *Bull World Health Organ.* **81:** 236-236.
- [8] Kang G, Arora R, Chitambar SD, Deshpande J, Gupte MD and Kulkarni M. 2009. Multicenter, hospital-based surveillance of rotavirus disease and strains among Indian children aged <5 years. *J Infect Dis.* **1:** 47-53.
- [9] Ramani S and Kang G. 2007. Burden of disease & molecular epidemiology of group A rotavirus infections in India. *Indian J Med Res.* **125:** 619-632.
- [10] Banerjee I, Ramani S, Primrose B, Moses P, Iturriza-Gomara M and James J. Gray. 2006. Comparative study of the epidemiology of rotavirus in children from a community-based birth cohort and a hospital in South India. *J Clin Microbiol.* **44 (7):** 2468-2474.
- [11] Swapin J, Nutan T, Neelam G, Jitendra V and Harish C. 2016. Prevalence of rotavirus, norovirus and enterovirus in diarrhoeal diseases in Himachal Pradesh, India **27:** 77-83.
- [12] Nguyen VT, Van VL, Huy LC and Weintraub A. 2004. Diarrhoea Caused by Rotavirus in Children Less than 5 Years of Age in Hanoi, Vietnam. *J Clin Microb.* **42:** 5745-5750.
- [13] Musawi Ma, Zainaldeen H, Shafi F, Anis S and Deantonio R. 2013. Rotavirus gastroenteritis in children under 5 years in the Kingdom of Bahrain: hospital-based surveillance. *Clin Epidemiol.* **5:** 269-275.
- [14] Chakravarti A, Chauhan MS, Sharma A and Verma V. 2010. Distribution of human rotavirus G and P genotypes in a hospital setting from northern India. *Southeast Asian J Trop Med Public Health.* **41:** 1145-1152.
- [15] Sanjay C. Chavan, Agarkhedkar S, Dipali S. Chavan, RP. Nagdawane and Smita Singhania. 2013. Prevalence of rotavirus diarrhoea among children hospitalized in a tertiary care hospital in Western India *Int J Pharm Biomed Sci.* **4 (1):** 4-7.
- [16] Bahl R, Ray P, Subodh S, Shambharkar P and Saxena. 2005. Incidence of severe rotavirus diarrhoea in New

Delhi, India, and G and P types of the infecting rotavirus strains. *J Infect Dis.* 1 **192**: 114-119.

- [17] Nath G, Singh SP and Sanyal SC. 1992. Childhood diarrhoea due to rotavirus in a community. *Indian J Med Res.* 95 (4): 259-262.
- [18] Phukan AC, Patgiri DK and Mahanta J. 2003. Rotavirus associated acute diarrhoea in hospitalized children in Dibrugarh, north-east India. *Indian J Pathol Microbiol.* **46**: 274-278.