Original research article

Prevalence of Rotavirus in Children in and Around Bihar

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Abstract

Background and objectives: Rotaviruses are the major cause of gastroenteritis in infants and young children worldwide. In India the mortality and economic burden associated with rotavirus is high. The purpose of the present study was to know the prevalence of rotavirus diarrhea in children in and around Bihar.

Material and methods: The study was conducted in Department of Microbiology, IGIMS, Patna. Study duration of Two years. The study included 120stool samples from pediatric patients of age group 6 months to 5 years. The samples were tested for the presence of rotavirus antigen using ELISA, (Enzyme linked immunosorbent assay) latex agglutination (LA) and immunochromatography (ICG) test.

Conclusion: Rotavirus infection was seen in a considerable proportion of infants and children in present study. Latex agglutination and immunochromatography test werefound to have good sensitivity and specificity, when compared with ELISA, for detection of rotavirus antigen. These tests can be used for screening cases of suspected rotavirus diarrhea during an epidemic outbreak.

Keyword: Rotavirus; Prevalence; ELISA; Latex agglutination; Immuno- chromatography test.

Introduction

Diarrhea remains one of the most common illnesses of children worldwide [1, 2]. In developing countries, it is the third most common cause of death with an estimated number of 2 million deaths per year or 17% of all deaths in children younger than 5 years of age .[3] In developed countries, diarrhea is the second most common cause of hospital admission and doctor visits. [3]

Although more than 20 different microorganisms (bacteria, parasites and viruses) cause

diarrhea; one pathogen, rotavirus, is the most frequent causative agentfor the most severe disease in children younger than 5 years of age worldwide. [3] Rotavirus causes 25-55% of all hospital admissions for diarrhea and more than 0.6 million deaths every year. [1]

Rotavirus is also a frequent cause of nosocomial infection in children who arehospitalized for reasons other than rotavirus gastroenteritis (RVGE). [4] Rotavirus affects virtually all children worldwide by the age of 5 years. [2] Eight humanserotypes have been identified, with serotype-1 being the most common. [6] Astrovirus infection accounts for 7-15% of infantile diarrhea in a variety of settings. Most infections occur in infants < 1 year of age. [7, 8] Sapovirus It is a synonym for "typical" human calciviruses, e.g., Sapporo virus, which was first isolated from Sapporo, Japan. Sapoviruses are known primarily for sporadic cases of diarrhea in children, although outbreaks in a closed population do occur. [9, 11] Adenovirus Adenoviruses are non-enveloped DNA viruses, 65 to 80 nm in diameter. The virion is roughly spherical in the form of an icosahedron. [6] Infantile diarrhea has been associated with epidemic and sporadic adenoviraldisease. Diarrhea outbreak characterized by acute abdominal pain followed by diarrhea, nausea, vomiting, fever and headache, have been associated with type 3 and7 infections. [12] Type 40 and 41 are called as enteric adenovirus. In enteric adenoviral infection, diarrhea is the most prominent symptom. Enteric adenovirus causes approximately 2 to 12 % of all diarrhea episodes in young children. [10] Coronavirus: It has ssRNA genome and helical capsid with envelope. The prefix corona is used because of the crown like surface projections that are seen under EM. Coronaviruses are thought to cause diarrhea in infants based on the presence (using EM) of coronavirus like particles in stool of symptomatic patients. [13]Torovirus:Toroviruses are 100-140 nm, enveloped positive strand RNA viruses. A study from Canada demonstrated an association between torovirus excretion andnosocomial GE in pediatric patients. [10]Rotavirus: Most common cause of diarrhea worldwide is rotavirus infection. Rotavirus belongs to family Reoviridae. The family Reoviridae derives its name from the prototype virus which was known as Respiratory Enteric Orphan virus, because it could be isolated frequently from the respiratory and enteric tracts. Members of this family are double shelled icosahedral viruses. The genome consists of double stranded RNA in 10-12 pieces. They are non-enveloped.

Objectives

To determine the presence of rotavirus antigen in stools of children (6 months– 5 years) suffering from acute gastroenteritis (AGE).To know the prevalence of rotavirus infection in children in our place. To compare latex agglutination test with ELISA test for detection ofrotavirus antigen.

Review of Literature

Viruses with morphologic features later associated with rotaviruses, were firstobserved (by Adam and Kraft) by electron microscopy in 1963 in intestinal tissues and rectal swab specimens from mice and monkeys. These agents, called as epizooticdiarrhea of infant mice viruses and simian agent 11, respectively, were described as 70 nm particles that had a wheel like appearance. [13] In 1969, these particles were demonstrated by Mebus and Colleagues in the stool of calves with diarrhea, thus associating these viruses with diarrheal diseases in cattle. [13,14] In 1973, Bishop and Colleagues reported the correlation between

these calves' viruses and human diarrheal disease. They used EM to examine biopsy specimens of duodenal mucosa from children with AGE in Melbourne. [5] Shortly afterwards rotavirus was identified in feces by using EM by Flewett etal, Bishop et al and others. [16] Later the human rotaviruses along with their animal rotavirus counterparts were shown to share a group antigen and have been classified as members of rotavirusgenus within the Reoviridae family. [15] In 1980, particles that were indistinguishable morphologically from established rotaviruses strains but lacked the common group antigen were discoveredin pigs. This finding subsequently led to the identification of rotaviruses belonging tosix additional groups (B to G) based on a common group antigen, with the original rotaviruses strains classified as group A. [16] Only group A to C have been associated with human diseases. Most commoncausative agent is group A. [6] Rotaviruses are stable to heat at 50^0C, to a 3.0 – 9.0 range of pH, and to lipidsolvents such as ether and chloroform, but are inactivated by 95% ethanol, phenol andchlorine. [6] Rotaviruses are classified by a scheme of groups and multiple serotypes/genotypes within each group.

Groups / Serogroups: Rotaviruses are classified into seven different groups (A – G), based on the antigenic specificity of the VP6 capsid proteins, as well as on the pattern of electrophoretic mobility of the 11 RNA segments of the viral genome. [15,16 Groups A, B and C rotaviruses are found in both humans and animals. [2Whereas rotaviruses of groups D, E, F and G have been found only in animals to date. [16Severe life threatening disease in children worldwide is caused predominantlyby Group A rotavirus. [15, 216 Subgroups (SG): Within Group A, four different subgroups (SG); SG I, SG II, SG I and II andnon I/ non II, have been distinguished on the basis of VP6 diversity, of which humanstrains are possibly only from SG I or SG II. [15, 18], Serotype: VP7 and VP4 comprise the outer layer of virion and are the basis of a binary classification system defining G types (Glycoprotein) and P types (Protease sensitive), respectively .[18,20] At present, 11 of 15 G types i.e., VP7 variants and 12 of 26 P types, i.e., VP4variants are known to infect humans. [3]

Material and methods

The study was conducted in Department of Microbiology, Indira Gandhi Institute of medical sciences, Patna, Bihar. Study duration of Two years. The study included 120stool samples from pediatric patients of age group 6 months to 5 years. The samples were tested for the presence of rotavirus antigen using ELISA, (Enzyme linked immunosorbent assay) latex agglutination (LA) and immunochromatography (ICG) test.

Specimen Collection:

During the period of diarrhea, stool samples were collected in a sterile bottle and transported to the microbiology laboratory as soon as possible.

It's a solid phase sandwich type EIA. Plastic micro-titer wells are coated witha monoclonal antibody directed against the group specific antigen (coded by VP_6 gene) for all known human rotaviruses. An aliquot of fecal suspension is added to the well and incubated simultaneously with an anti-rotavirus monoclonal antibody conjugated to horse-radish peroxidase, resulting in the rotavirus antigen being sandwich between the solid phase and enzyme linked antibodies. After 60 minutes incubation at room temperature, sample well

is washed in order to remove unbound enzyme labeled antibodies. Enzyme substrate A (urea peroxide) and substrate B (TMB) are added to the wells and incubated for 10 minutes at room temperature. Theenzyme bound in the wells converts the colorless substrate to a blue color. The intensity of the blue color is directly proportional to the concentration of rotavirus antigen in the sample.

Procedure:

Sufficient numbers of wells were snapped off (for samples and controls) and wereinserted into the micro-titre well holder. Sample positions were recorded. 100µl of diluted fecal sample positive control and negative control (samplediluents) were added to the bottom of separate wells.

1. 100 µl of enzyme conjugate was added to each well. Contents in well were mixed well by gently swirling on table top.
2. Wells were incubated at room temperature for 60 ± 5 minutes.

Results can be determined visually or by spectro-photometeric procedure:

1. Positive result by visual determination – samples which showed blue color more intense than that of the negative were considered positive (after incubation period of step 10 was over). Samples with color equal or less intense than the negative control are negative.
2. Positive results by spectro-photometric determination – after step 11, absorbance value for each well was read at 450nm using a > 600nm reference filter against an air blank within 60 minutes.
3. Rotavirus Immunochromatography Test (One step Rotavirus Antigen Test) Principle:
4. This test utilizes two kinds of antibody in a solid phase sandwich immunochromatography to detect group specific proteins, including the major inner capsid protein, present in Group A rotavirus. Immunochromatography Cassettes (I) Negative Sample and (II) Positive Sample

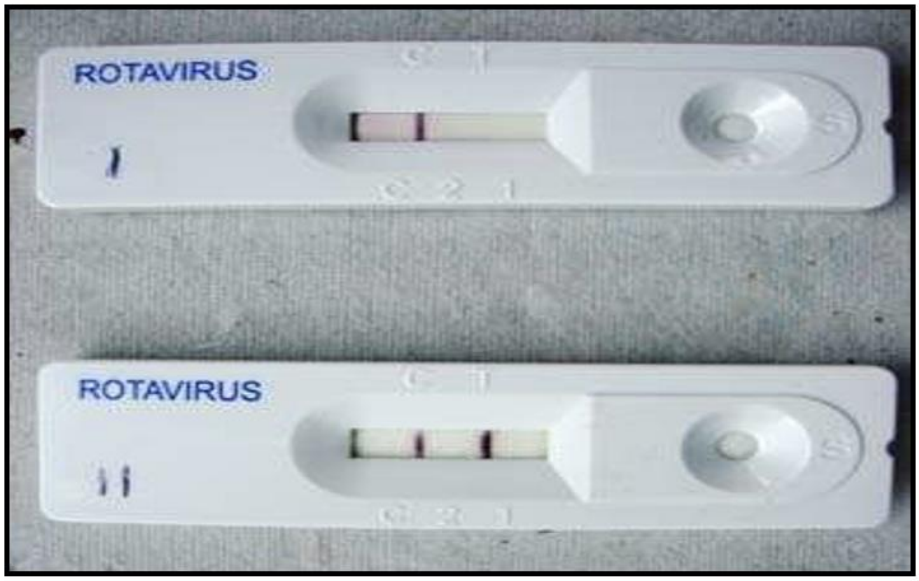

Figure 1:

Results

Stool sample was collected from children from six months to five years of age fulfilling the selection criteria. The study included 120 children suffering from diarrhea in age

group of 6 months -5 years.

Table 1: Age and Sex Distribution of Diarrhea Cases

Age	Males	%	Females	%	Total	%
6 – 12 Months	26	21.66	14	11.66	40	33.33
1 – 2 years	24	20	16	13.33	40	33.33
2 – 3 years	14	11.66	8	6.66	22	18.32
3 – 4 years	6	5.00	4	3.33	10	8.33
4 – 5 years	4	3.33	4	3.33	8	6.66
Total (6m-5years)	74	61.66	46	38.33	120	100

Table 2: Age and Sex Distribution of Rotavirus Positive Diarrhea Cases

Age	Males	%	Females	%	Total	%
6-12 Months	5	20.83	3	12.5	8	33.33
1-2 years	7	29.16	5	20.83	12	49.99
2-3 Years	2	8.33	0	0	2	8.33
3-4 years	1	4.16	0	0	1	4.16
4-5 years	0	0.00	1	4.16	1	4.16
Total (6m-5years)	15	62.5	9	37.5	24	100

Total number of rotavirus positive cases from 6 months to 5 years were 24. Maximum cases were seen in the age group of 6 months to 2 years (20).which was statistically significant. (Chi-square test (^{2}) = 7.86, p value < 0.05)

Table 3: Prevalence Rate of Rotavirus Detected with Three Types of Test

Test Done	Prevalence rate (%)
ELISA	20
Latex Agglutination	21.6
Immunochromatography test	20

Table 4: Total Number of Positive and Negative Cases Observed with Latex Agglutination as Compared to ELISA

		ELISA		Total
		Positive	Negative	
Latex agglutination	Positive	23	3	26
	Negative	1	93	94
Total		**24**	**96**	

Table 5: ELISA Enzyme linked immunosorbent assay LA-Latex agglutination Diagnostic Efficacy of Immunochromatography Test when Compared with ELISA

Sensitivity of ICG test	91.66%

Specificity of ICG test	97.91%
Positive Predictive Value of ICG test	91.66%
Negative Predictive Value of ICG test	97.91%
Accuracy	96.65%

Table 6: Together Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Accuracy for LA and ICG Tests Compared with ELISA

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**	**Accuracy**
LA	95.8%	96.87%	88.46%	98.93%	**96.66%**
ICG	**91.66%**	**97.91%**	**91.66%**	**97.91%**	**96.65%**

The majority of the cases occurred in children younger than 24 months, which is the susceptible expected target age group. In children more than two years of age, rotavirus infection can be asymptomatic, as local intestinal immunity owing to previous infection with rotavirus confers some protection against subsequent rotavirus infections, with repeated infections becoming less severe or asymptomatic. Previous studies from India as well as from different countries have revealed varying rates of prevalence of rotavirus infection that ranged from as low as 4 to 62.6%. These wide ranges can be due to the differences in age group studied, detection methods employed, time of onset and the seasonal variation of rotavirus diarrhea in different regions of the country. ELISA has been used in most of the studies for detection of rotavirus. ELISAis used widely in diagnostic laboratories because they provide rapid detection of rotavirus antigen in a relatively short time in comparison to other tests, such as virus isolation. The prevalence of childhood rotavirus in the north Indian cities of Delhi, Chandigarh and Aligarh has been reported to vary from 6-45 per cent. In the western states of India, in Pune, rotavirus was detected in 28-30 per cent of children under. 5 yr of age with acute diarrhea. In eastern India, in Kolkata the incidence of rotavirus associated diarrhea varied from 5-22 per cent. On the other hand, in Manipur the incidence was as high as 41 percent. Bahl R et al., found out rotavirus in 23.5% of stool sample of children suffering from diarrhea in New Delhi. The study done by Saravanan P et al., showed an overall infection rate of 22.55% among children with acute diarrhea.[17] Where as another study done at Vellore, found rotavirus in 27.4% of all diarrhea cases in children aged up to five years. [50] Kang et al., found a prevalence rate of 39% in a Multicenter, hospital-based surveillance of rotavirus disease and strains among Indian children aged <5 years. [22] Jain V et al., analyzed 40 published studies of rotavirus that were conducted between 1976 and 1997 and included a total of approximately 13,000 Indian pediatricinpatients. Rotavirus was detected in a median of 18% of pediatric patients. Across the world too prevalence of

rotavirus diarrhea varies from country to country. The Reveal Study done by Van Damme P et al., reported a prevalence rate of 40.6% across seven European countries. Raboni SM et al., where as another study done by Cardoso DDP et al., detected a prevalence rate of 11.8% in children suffering from diarrhea in Central Brazil. [18] Altindis M et al., detected rotavirus positive in 12.59% of stool specimens from 135 children of 0 to 3 years old.[23] Shariff M et al., detected a prevalence rate of 38.7% in Eastern Nepal.[24] Jain V et al., reported that rotavirus was most prevalent (31%) in children between 7 and 12 months of age, followed by children between 1 and 2 years of age (20%). Kang G too reported that rotavirus detection rates were greatest among children aged 6-23 months. [22] Shariff M, found out that the majority (70.9%) of infections were observed in patients between 6 months and 2 years of age in a study done in Eastern Nepal. [24] It has been observed that temperature influences the stability of human and animal rotaviruses that contributes to the efficient transmission of the human rotaviruses. Moreover the influence of low relative humidity in the home has been suggested as a facilitating factor for the survival of rotaviruses on surfaces. [18] Saravanan P et al., reported in their study which was conducted between 1996 to 1998 that the hotter months (March –August) in all the three years (1996, 1997 and 1998) had decreased rate of rotavirus associated diarrhea (17.6, 17.0 and 14.3% respectively) than the cooler months (September – February) (21.8, 31.8 and 22.5% respectively). The same pattern could be appreciated when the summation of seasons were viewed (16.4% in hot months and 25.9% in cool months). [218 In another study done by Kang et al., there was marked seasonal peak in rotavirus in northern temperate locations but was less seasonal in southern locations with a tropical climate. [22] Raboni SM et al., too observed a seasonal pattern in rotavirus diarrhea, which was associated more with cooler temperatures and a drier atmosphere. [20], Bahl R et al., reported that hospitalizations for rotavirus- associated diarrhea occur year-round in Delhi, but there was a distinct peak in winter (i.e., from November through February). [25] Similar trend was also observed by Shariff M et al., in Eastern Nepal where rotavirus infections occurred throughout the year with the peak in late winter (January- February), and to a lesser degree, during the summer/ monsoon months (April-August). [18] In contrast, Banerjee I et al., reported no significant seasonal trends in their study done in Vellore. However, they found a peak of rotavirus diarrhea in the months of July to September in 2003, corresponding to the rainy season. [26]

Conclusion

Rotavirus is most common cause of diarrhea in children world wide including developing countries. The present study too showed that rotavirus is an important pathogen of acute diarrhea in infants and children. Most of the laboratories used ELISA as the method of diagnosis of rotavirus infection.Development of sensitive enzyme immunoassay has made diagnosis of rotavirus infection easy and widely available. ELISA has the advantage to be the most sensitive and specific while Latex agglutination test has the advantage to be the quicker method.

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