Bangladesh J Med Microbiol 2012; 06 (01): 11-13
Bangladesh Society of Medical Microbiologists

Original Article

Rapid detection of Rotavirus antigen in stool sample of acute diarrheic children.

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Abstract

Rotavirus is one of the leading causes of pediatric diarrhea globally. Accurate and rapid diagnosis of Rotavirus diarrhea should reduce unnecessary use of antibiotics and ultimately reduce drug resistance. Study was designed for rapid diagnosis of Rotavirus antigen in stool sample by ICT (Immunochromatographic test) as well as to observe the seasonal variation of rotavirus infection. This cross sectional study was carried out in the department of Microbiology, Dhaka Medical College from January 2011 to December 2011. Eighty stool samples were collected from Dhaka Shishu Hospital and Dhaka Medical College Hospital. All samples were tested for rotavirus antigen by ICT. Among 80 patients, 42 (52.5%) samples were positive for rotavirus antigen. Among these 42 positive samples, 30 (71.43%) were from 0-12 months of age group, 10 (23.81%) from 13 to 24 months of age group and rest 2 (4.76%) from 25 to 36 months of age group. Rotavirus Ag was detected in stool samples from January to April and another peak episode from October to December. Considering the importance of Rotavirus associated diarrhea, rapid detection of Rotavirus infection in human is substantially needed and should be routinely practiced.

Keywords: Rotavirus, Diarrhea, Immunochromatographic test.

Introduction

Acute diarrheal diseases are a major public health problem leading to high morbidity in both developed and developing countries, with the additional burden of high mortality in the later. Rotavirus infection is the most common cause of severe diarrhea in young children leading worldwide[1]. Almost all kids have had a rotavirus infection by the time they are 5 years old. It is estimated that rotavirus infection annually causes 111 million episodes of gastroenteritis requiring home care, 25 million clinic visits, 2 million hospitalizations and approximately 600,000 deaths in children under 5 years of age[2].Three of the seven rotavirus groups are known to infect the humans. Among them, the most dominant is group A which causes diarrheal diseases worldwide[3]. In Bangladesh, rotaviruses cause 6,000-14,000 deaths each year in children <5 years of age[4]. WHO estimated out of 73% yearly death of children younger than 5 years; of which diarrhea was responsible for 18% of total deaths[5].

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Against this background of morbidity and mortality there is a need for rapid and sensitive rotavirus detection methods in routine diagnostic laboratories, many of which perform rotavirus group antigen detection using either enzyme immunoassay (EIA) or latex agglutination assay[6].It is noteworthy that conclusive evidence of viral infections is direct virus detection by electron microscopy. Although rotavirus can be isolated from stool sample by culture, but it is a cumbersome process and needs equipped laboratories and skilled personnel. The recent advent of antigen detection methods based on immunological techniques using polyclonal or monoclonal antibodies has gained the attention of researchers. Direct detection of antigen in stool samples by rapid one-step assay is an inexpensive, easy to handle sensitive test with no need of invasive procedures and specialized instrumentation [7].

Material and methods

A cross sectional study was conducted from January, 2011 to December, 2011. Stool samples were collected from 80 children (under 5 years) of acute diarrhea from both outpatient and in patient departmentsof Dhaka Medical College Hospital and Dhaka Shishu Hospital. All stool samples were collected in a dry, clean, leak proof plastic container and brought to the

microbiology laboratory of Dhaka Medical College within 2 to 4 hours. Cases were defined as children whose main complaint was acute diarrhea, characterized by the occurrence of three or more loose, liquid or watery stools with or without mucus in a 24 hours period.

Stool samples were analyzed for group A human Rotavirus by using an immunochromatographic test (ICT) (SD BIOLINE Rotavirus test, Standard Diagnostic, Inc. Korea). This test was a qualitative test based on association of monoclonal antibodies (nitrocellulose-based membrane pre-coated with rabbit monoclonal anti-Rotavirus antibody and the specially selected monoclonal anti-Rotavirus antibody are used as detector materials) specific to major inner capsid protein present in group A Rotavirus.

The statistical significance of the data was examined by chi square test (x^2) and probability value (P) < 0.05% was regarded as statistically significant.

Rusults

A total of 42 (52.5%) cases from the hospital were human rotavirus antigen positive. Table I represents the age distribution of children with rotavirus diarrhea. No positive cases were observed older than 36 months. Sex distribution of children with Rotavirus diarrhea is shown in Table II. The seasonal variation of rotavirus infection was also determined (FigureI).

Table-I: Age specific detection rate of rotavirus antigen among study population (n=80)

Age (Months)	Positive N (%)	Negative N (%)	Total N (%)
0-12	30 (71.43)	10 (26.32)	40 (50.00)
13-24	10 (23.81)	12 (31.57)	22 (27.50)
25-36	2 (4.76)	6 (15.79)	8 (10.00)
37-48	0 (0.00)	4 (10.53)	4 (5.00)
49-60	0 (0.00)	6 (15.79)	6 (7.50)
Total	42 (100)	38 (100)	80 (100)

p<0.05, statistically significant for 0 to 12 months of age group.

Table-II : Sex distribution of Rotavirus Ag positive cases (n = 42)

Age groups	Male N(%)	Female N(%)	Total N(%)
0-12	20 (66.67)	10 (33.33)	30 (100)N= 58
13-24	7 (70)	3 (30)	10 (100)
25-36	2 (100)	0	2 (100)
37-48	0	0	0
49-60	0	0	0

p>0.05.So, statistically not significant.

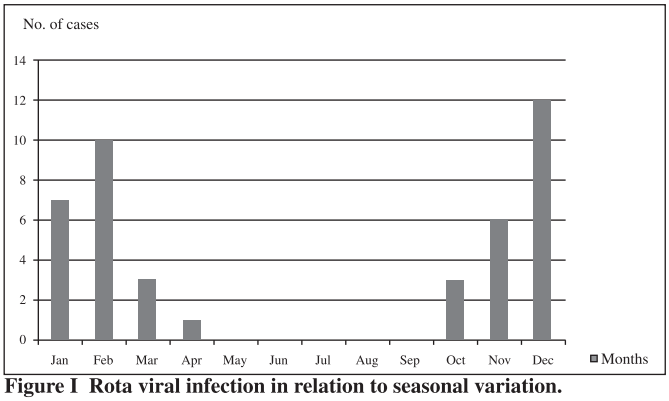

Figure I Rota viral infection in relation to seasonal variation.

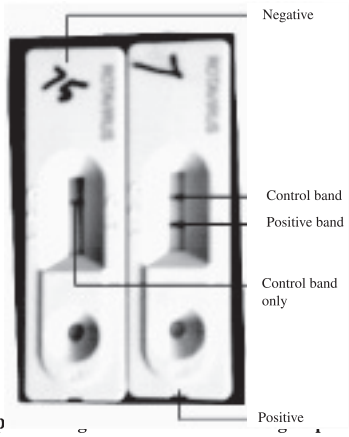

Figure II Photograp Rotavirus antigen.

Discussion

Rotavirus is the leading agent of hospitalization due to diarrhea among children through the world[8]. Detection of the rotavirus in diarrhea is necessary for estimation of itsfrequency within a community. This is particularly contextual for Bangladesh, where diarrhea is still contributing a significant proportion of mortality and morbidity in under five children and where rotavirus diarrhea is claimed to be on the rise[9].

Among these 80 samples, 42 samples werepositive of rotavirus antigen. Rotavirus antigen was detected in 42 (52.5%) cases. Thirty (71.43%) and 10 (23.81%) were from 0 to 12 months and 13 to 24 months of age groups respectively and this finding was statistically significant (p<0.05). In Bangladesh, 38.5% of children were suffered from rotavirus infection and among them, 67% were from first year of life which coincides with the present study[10]. Similarly ICDDRB reported that rotavirus was detected in 33% children with diarrhea and of them, most were among children age of 3-24 months[11].In a study in Bangladesh, 41.8% of the stool samples were positive for rotavirus and around 90% patients were within the first two years of life[12]. Similarly, In China, 40.9% were affected by rotavirus and it was the major cause

of diarrhea of infants and young children[13].In India, 48.1% children were suffered from rotavirus infection and of them 53.1% and 59% were from 0 to 11 months and 12 to 23 months of age groups respectively[14]. In contrast to this study, 21.88% and 17.2% of the samples were positive for rotavirus antigen in Italy[15-16].This difference may be due to seasonal variation and geographical areas.Rotavirus detection rate between genders showed 29 (58%) cases were rotavirus positive out of 50 samples of male patients and 13 (43.3%) out of 30 samples of female patients. This difference was statistically not significant. But no plausible explanation is imparted in this event. Similarly Nguyen in Vietnam, have shown that rotavirus infection is more frequent in male than female [17].

Detection of the rotavirus in diarrhea is necessary in assessing the clinical severity as well as providing an estimate of rotavirus diarrhea within a community. Accurate and rapid diagnosis of rotavirus diarrhea should reduce unnecessary use of antibiotics and ultimately reduce antibiotic resistance. Rapid rotavirus diagnostic capacity imparts a quick turn around time and improving infection control and nosocomial outbreaks. This is particularly important in Bangladesh, where diarrhea is still contributing a significant proportion of mortality and morbidity in under five children.

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