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RESEARCH ARTICLE

SCREENING AND IDENTIFICATION OF HUMAN ROTAVIRUS BY ELISA AND RNA-PAGE METHOD

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ABSTRACT

Rotavirus is causative agent of diarrhea resulting in mortality in children up to the age group of 5 years. The first infection occur between age group of 6-9 months. However, the need for screening of rotavirus is important to access the incidence of severe disease or effectiveness and its serotype distributions. To detect any new ideotype in re-assortment a continuos screening of existence genotype is required. Multiple assay now exist for measuring the serological response to Rotavirus infection. ELISA and RNA-PAGE are considered to be highly sensitive tool for screening of Rotavirus because its abilty to detect even at low concentration.

INTRODUCTION

Rotavirus is the major cause of severe dehydrating diarrhea and a leading cause of mortality in children. It is the one of the most common disease in this age group in to the world. More than 6,00,000 deaths occur annually into the children under the age group of 5 years due to the gastroenteritis infection (Parashar *et al*., 2006). Into the developing countries it constitutes the major cause of the morbidity and mortality into the children. Rotavirus infection has a short incubation period of between 1 to 3 days. It is mainly characterized by the onset of acute watery diarrhea often accompanied by fever and vomiting. Rotavirus belong to the *Reoviridae* family and Sedoreovirinae sub-family. Rotavirus contains double stranded RNA and genome of 11 segments. It encode by six structural; and six- non structural protein. The diameter of rotavirus is 70-75 nm. Three paired of icosahederal protein capisd layer contain core, inner capsid and outer capsid. Rotavirus are classified in seven different serogroups (A-G) based on to their antigenic specificities of the capsid protein. The inner most capsid are composed of VP6 protein and the outer most capsid contain glycoprotein VP7 (G-serotype) and the spike protein VP4 (P- serotype). At least 15 different G-serotype and 26 different P-serotype have been found in human and animal infection. Of the seven serogroups only groups A, B and C are known to human infect in human and group A virus are those that cause severe, life threatening disease in children in world wide. VP7 and VP4 elicit neutralizing antibodies.

The incidence and the distribution of group A rotavirus serotype and genotype vary between geographical area during a rotavirus season and from one season to the next. Globally G1-G4 and P1A [8] and P1B [4] are the most common in G and P type causing disease in humans. The reverse transcriptase PCR helped in identification of strain diversity with at least 42 P-G combination being recognized in human infection and also helped in identification of additional globally (G9) and regionally (G5, G8, P2A[6]) common strain that are not covered by the vaccine that have undergoes trials (Kang, 2006). Rotavirus diversifies and evolves mainly by two mechanisms: First is the accumulation of the point mutation which generates genetic lineage lead to emergence of antibody escape mutants. The second thing is genetic shift, in which exchange of genetic material through gene-rearrangement occurs during infection of a single cell. The diversity and capacity of human rotavirus for rapid evolution and reassortment suggest that rotavirus vaccine must provide good heterotype protection to be effective in different geographic regions.
Testing of rotavirus is most helpful to eliminate other treatable causes of diarrhea that are caused by bacteria and parasites or if the diarrhea is lasting longer than expected. Children do not develop complete immunity to the rotavirus infection, of which there are several subtypes, so can get infected more than once, although repeat infection are usually milder than the first one. The genome of this virus is segmented in nature, so there are chances of reassortment between these segments. This reassortment process may give rise to a completely new genotypic combination against which existing vaccine may be ineffective. To detect the presence of new reassortment viruses, a continuous surveillance of existing genotype is required. This epidemiological surveillance will be vital for incorporating any new ideotype in multivalent vaccine preparation.

MATERIALS AND METHODS

Collection of fecal samples

A total of fifty two stool samples were collected over a period of approximately four months from human between age group of 0-5 years, admitted with acute diarrhea at the following Rohtak.

1. Dept. of Pediatrics, PGIMS, Rohtak.
2. Hooda clinic, Gohana stand Rohtak.

All the stool samples were collected in screw capped plastic vials and store at 4°C. The samples were later transferred to laboratory in ice and store at -20°C till further processed.

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Sample Processing

The stool samples were diluted in lysis buffer to make a 10% suspension, followed by centrifugation at 10,000g for 15 min. to remove coarse particles and cellular debris. The clarified supernatants were store at -20°C till further use ELISA and RNA-PAGE.

Rota clone (Enzyme Immune Assay) protocol

EIA for the detection of rotavirus Ag in human fecal sample. This is based on detection of VP6 antigen with the help of polyclonal antibody. This is most commonly used test for detection of rotavirus in routine. Protocol for EIA was followed according to Premier™ Rota clone kit of Meridian Biosciences Inc., USA. Spectrophotometric analysis was made by adding 100 µl of stop solution (sulfuric acid) in each well. Each well at 450 nm using >600 nm reference filter against air blank.

Extraction of viral RNA from faecal sample

The viral RNA was extracted from faecal sample having Rotavirus by sodium dodecyl sulphate (SDS)-phenol-chloroform method (Rio et al., 2010.)

RNA-Polyacrylamide Gel Electrophoresis

RNA-Polyacrylamide Gel Electrophoresis was run for screening and identification acoording to protocol of Rio et al., 2010.

RESULTS

Screening of diarrheic fecal sample by the ELISA method

An aliquot of sample was taken for testing by the ELISA method. A total of 52 fecal samples were screened from the diarrheic children in the region around the Rohtak area. Out of 52 fecal samples 14 sample were found positive for rotaviruses infection. All positive fecal samples exhibit sky blue color in ELISA method. (Figure 1,2).

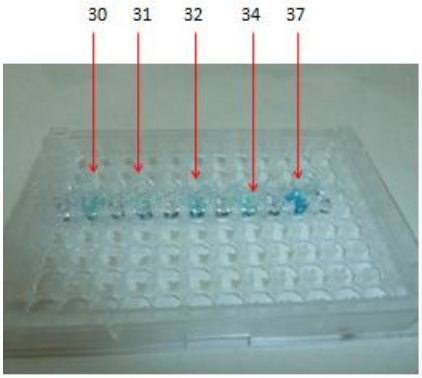

Figure 1. Testing of diarrheic faecal sample by ELISA

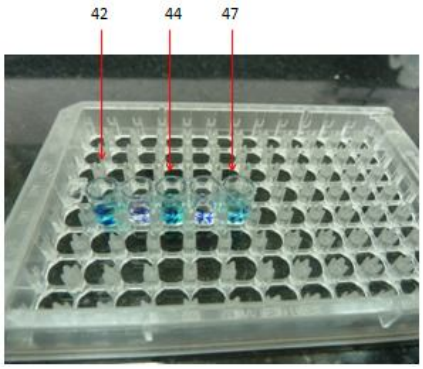

Figure 2. Screening of sample no. 42, 44 and 47 by the method of the Enzyme immunoassay

ELISA revealed classical blue colors with their positive sample. Incidence for human rotavirus was found to be 26.92%. Positive and Negative control reaction were also taken out to exclude the possibility of false positive and false negative results. Strong positive result in positive control and no color developed in negative control authenticate the testing of sample by ELISA kit.

Screening of the rotavirus from the diarrheic patients by the RNA-PAGE method

All the 52 diarrheic fecal sample were subjected to RNA extracted by SDS- PCI method. RNA pellet was dissolved in triple glass distilled water and loading was done after mixing each extracted sample with 1X loading dye. Out of the 52 RNA sample only 14 samples exhibit the typical pattern of migration. The positive faecal samples exhibit RNA size based migration in polyacrylamidegel (Fig. 3). RNA-PAGE revealed classical 11 segments with 4:2:3:2 migration patterns. Incidence for human rotavirus was found to be 26.93%. All RNA-PAGE positive samples were of long electrotype because of faster migration of the 11th segment relative to 10th segment (Fig.4).

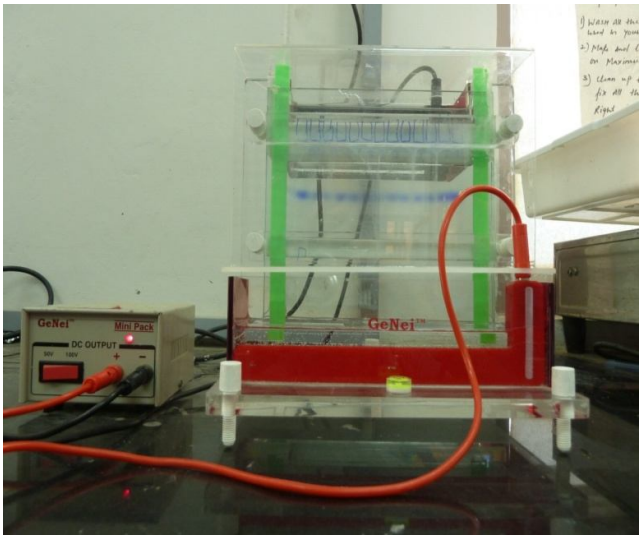

Figure 3. GeNei midi electrophoretic assembly used for RNA-PAGE

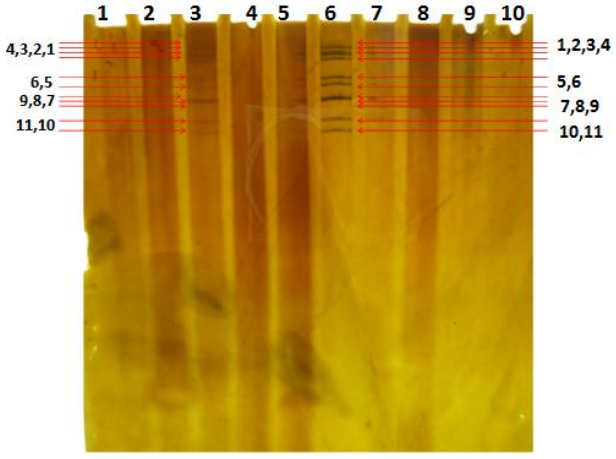

Figure 4. Electropherotypic profiling of Rotavirus genome on non-denaturing polyacrylamide gel

Lane3: HRV; 30
Lane6: HRv; 34

In some sample the segment no. 10 and 11 moved separately from the segment no. 7, 8 and 9.

Another classical revealed 11 segment with 4:2:3:2 migration patterns. All of the positive sample migrate long electrotype because of the faster migration of the sample 8 and 9 as compare to the 4 and 6. Lane 1:- HRv; 1 (sample 37), Lane 2:- HRv; 2 (sample 42), Lane 3:- HRv; 3 (sample 44), Lane 4:- HRv; 4 (sample 47). In sample no.42 and 47 segment 10, 11 appear as one band and 7, 8, 9 appears as one band. (Fig 5.)

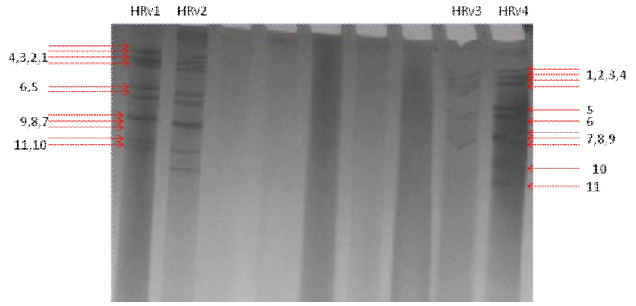

Fig.5. Electropherotypic profiling of Rotavirus genome on non-denaturing polyacrylamide gel.

DISCUSSION

In this study, group A rotaviruses were detected in 26.92% of the stool samples of children less than five years with GE. This 4-month survey of enteric virus infection in and around Rohtak indicates that rotaviruses were circulating within the community throughout the year, with infection peaking in the cooler and drier months of July (40%) 8 sample found positive out of the 20 and August (17.64%) 6 sample are positive out of the 34. Published data indicated that the highest rotavirus prevalence rates occurred in children aged 6-18 months with severe infections occurring more frequently in younger children aged 6-12 months in developing countries. In addition, infection often occurs in children less than three months of age. A Venezualan study by Salinas *et al.* (2005) reported the generation of rotavirus-specific IgA antibodies following rotavirus infection. Some Rotavirus strains appear to be transmitted to a different species as a whole genome, these data suggest that interspecies transmission may occur frequently in nature (Fujiwara and Nakagomi, 1997; Iizuka *et al.*, 1994; Nakagomi and Nakagomi, 2002; Nakagomi *et al*., 1990; Palombo, 2002; Matthijnssens *et al.*, 2006; Tsugawa and Hoshino, 2008; Nguyen *et al.*, 2007; Parra *et al.*, 2008; De Leener *et al.*, 2004).

The detection of G8 strain in Delhi region (India) with the sequence variation in strain of the G and P genotypes was reported (Sharma. *et al.*, 2009). This study carried out would also helps to detect novel strain that may emerge in the local community and also regularly updated our typing method with the addition of the new primers. Other methods of diagnosis of rotavirus infection depend upon the detection of viral genomic RNA. These methods include direct visualization of rotavirus genomic RNA by PAGE, DNA probe hybridization assays and RT-PCR. Molecular techniques, such as electropherotyping and PCR, have become widely accepted as the assay of choice for the fast and complete characterization of field isolates. The unique feature of the ds RNA of the rotaviruses and their genomic encoding information for the structural and non-structural proteins and applied polyacrylamide gel electrophoresis (RNA-PAGE) (Mattion *et al.*, 1994). Similarly finding was reported by Aijaz *et al*., 1996, recorded prevalence of 21.60% in Bangalore and only 11.50% in Mysore. Saravanan *et al.*, 2004 reported occurrence of 22.5 % among children in Chennai. Similarly in Vellore, Brown *et al.* 1988 recorded 18% prevalence among children.

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