RESEARCH BRIEF

A Unique Human Rotavirus (non vaccine) G9P4 Genotype Infection in a Child with Gastroenteritis

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Group A Rotaviruses with serotypes G1-G4 and G9 are the common Rotavirus types of clinical importance. This study aimed at determining the different Rotavirus genotypes in stool sample of children below 5 years. A total of 300 children with acute gastroenteritis were tested for group specific VP6 antigen of group A Rotaviruses by Enzyme Linked Immunosorbent Assay. 47 of these samples were positive for Rotavirus antigen. Out of these, 20 positive samples were subjected to Reverse Transcriptase Polymerase Chain Reaction for genotyping. The identified genotypes were G9P8, G1P8, G2P4, G9P4 (non-vaccine genotype), G1P6, and G1 (P types not identified in 5 samples).

Key words: *Genotypes, Rotavirus, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).*

The studies on epidemiological profile of rotavirus infection have revealed that 20-50 percent of hospitalizations for diarrhea among children in 0-5 years age group are associated with this infection. The morbidity and mortality are much in children infected with rotavirus than previously estimated, and is responsible for two million deaths per year in developing countries [1-3]. Rota virus genogroups are based on the structure of specific genes: G types (G1-G14) refer to the VP7 protein (gene 9) and P types P1A[8]-P1B[4] refer to VP4 protein (gene 4) and are differentiated by Reverse transcriptase polymerase chain reaction (RT-PCR). Rotaviruses can therefore be typed and strains circulating in the community can be monitored. The strains change in frequency of circulation over time, and occasionally new reassortment strains are introduced to a community [4,5]. At least 42 P-G combinations have been recognized in human infections. Some of the vaccine strains like G9, G8, G5 and P2A[6] are not covered by available Rota virus vaccines that have undergone clinical trials[6]. This study is part of an ongoing research project in a tertiary care hospital.

METHODS

A total of 300 stool samples were collected from children under 5 years of age. These children were admitted for symptoms of diarrhea, vomiting, abdominal pain and dehydration due to acute gastroenteritis in Department of Paediatrics, SRM Medical College Hospital and Research Centre, Kattankulathur village, Tamilnadu during the period January 2009-December 2010. Immediately after their admission, we collected 5 to 10 mL of fresh stool sample from the patients, in a sterile container.

The laboratory request form with all necessary details of the patient was duly filled up. An informed consent form signed by the guardian was obtained. The Institutional ethical committee clearance was obtained for this study. Stool samples were stored in sterile vials containing 70 percent tryptic soya broth with 30 percent glycerol, labelled with patient details and kept in deep freezer at -20° Celsius for long term use.

Three months after adequate number of stool samples had been collected, we performed Enzyme Linked Immunosorbent Assay (ELISA-GA Generic assays GmbH, Germany). Rotavirus antigen is a fast enzymometric one- step immunoassay for the qualitative determination of Rotavirus antigen, employing a solid phase immobilized polyclonal antibody (sheep) and murine monoclonal antibodies conjugated to horseradish peroxidase. Both antibodies are directed against the group specific VP6 antigen of group A rotaviruses. The test was performed with the positive and negative control, and assay was performed according to the manufacturers protocol. Optical density (OD) of the solution read at 450nm is directly proportional to the amount of rotavirus antigen bound. For optimal results, a reference filter of 620nm wavelength was used. Recommended cut-off value results were interpreted as positive or negative.

Diagnostic RT PCR was done for the 47 samples that were positive for rota viral antigen. Extraction of RNA was performed using QIAGEN miniviral RNA kit (QIAgen Gmbh Hildem, Germany) in accordance with manufacture's instructions. The double standard RNA (dsRNA) was extracted directly from faecal sample. Prior to addition of RNA to RT-PCR master mix, sample RNA was subjected to denaturation at 95° C for 5min followed by incubation in ice for 2min, to separate the Rotavirus dsRNA. Single stranded RNA was used as template for RT-PCR to amplify the VP6 antigen coding gene (VP6F-GACGGVGCRACTACATGGT and VP6R-CCAATTCATNCCTGGTG.) by using INVITROGEN one step RT-PCR Kit [7].

20 samples of rotavirus antigen positive by ELISA and RT-PCR were subjected for P and G genotyping with appropriate primers, and partial nested RT-PCR was performed to determine both the VP7 (G) and the VP4 (P) genotypes. For VP7 genotyping, rotaviral RNA was subjected to RT-PCR with primers VP7-F,VP7-R followed by 35 cycles of RT- PCR (each at a concentration of 20 µM). For VP4 genotyping, all procedures were identical to those described above, except that RT and the first amplification was done with appropriate primers. Genotyping was done using multiplex PCR for (VP7) G-Type and (VP4) P-Type. The samples were then resolved on 1 percent agarose gel to determine the G and P types. This combined typing scheme was designed to detect VP7 genotypes G1, G2, G4, and G9 as well as VP4 genotypes P4 and P8.

RESULTS

47 stool samples were positive for rotavirus antigen by ELISA and all of these were positive by RTPCR (positive predictive value for the test was 100 percent). The different genotypes identified in 20 positive samples were G9P8, G1P8, G2P4, G9P4, G1P6 and G1(P- type was not identified in 5 samples) (***Table* I**). To the best of our knowledge, the genotype G9P4 is a unique rotavirus strain that has not been reported previously from India.

DISCUSSION

The majority of the children who tested positive for rotaviral antigen had clinical features of vomiting and moderate dehydration. Rotavirus antigen detection by ELISA and RTPCR for routine screening is labour intensive and has high cost implications. Since the number of virus particles will decrease beyond acute phase of gastroenteritis, we collected stool samples within 3 days after onset of symptoms (to avoid false negative results).

TABLE I AGE DISTRIBUTION OF CHILDREN WITH DIFFERENT GENOTYPES OF ROTAVIRUS IN STOOL SAMPLES

Genotype	*Total no. of cases*	*Age group*			
		< 1 yrs	*1-2 yrs*	*2-3 yrs*	*3-5 yrs*
G9P8	1	–	1	–	–
G1P8	7	3	2	1	1
G2P4	5	2	–	1	2
G9P4	1	–	1	–	–
G1P6	1	1	–	–	–
G1*	5	3	1	–	1
Total	20	9	5	2	4

**P-not able to identify.*

The G9P4-non vaccine G serotype was identified in a one and half year old child. This child was admitted with severe dehydration due to diarrhea, vomiting and fever. Other routine laboratory investigations were not significant. The child was treated with parentral fluids and recovered within 3 days.

Previous studies done in India, during 1990-1991 in New Delhi had G1P8 strain as the predominant strain. It was documented that during the period of 1992-1993, G9P11 strain had emerged. In 2000-2007, a new strain of rotavirus namely G12 was identified [8]. This seems to indicate a notable relative shift in the prevalence of circulating viruses, which should be monitored over the coming years. In the same manner, while analyzing the emergence of new variants in Tamilnadu, South India, G1 strain were found in Vellore during 1983-1985 and G1-G2 P4-P8 in Chennai during 1995-1998 [9].

Currently there are two Rota virus vaccines available. One of them is a monovalent Rotavirus G1P8 vaccine while the other one is a pentavalent Rota Virus vaccine covering G1, G2, G3, G4 and P8. This study showed a case of G9P4 infection in a child. This serotype is not a part of the vaccine serotypes. This study revealed the considerable serodiversity among human rotaviruses in this geographical region and hence emphasizes the need to give protection with multivalent rota virus vaccines.

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WHAT THIS STUDY ADDS?

- A unique G9P4 non-vaccine genotype Rotavirus infection in a child caused acute gastroenteritis.

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