Detection of human rotavirus in hospitalized diarrheic children in central India

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Abstract During the present study, group A human rotaviruses were detected among diarrheic children using polyacrylamide gel electrophoresis (PAGE) technique, with a typical RNA migration pattern of 4:2:3:2, suggestive of group A rotavirus. During the study, a total of 46 fecal samples collected from hospitalized children with acute diarrhea as well as children inhabiting nearby animal farms with history of presence of animal rotaviruses on the farms were processed for detection of human rotavirus. Out of 33 diarrheic children, 12 showed presence of rotavirus infection (36.36%), however, none of the children from animal farm areas showed presence of rotavirus. Female children were more susceptible to rotavirus infection (46.15%) than males (30%). Majority of the cases of rotavirus gastroenteritis belonged up to one year of the age, with an incidence of 40.91%. RNA profile of rotaviruses suggested circulation of 5 different electropherotypes in this geographical locale of the country, indicating existence of genomic diversity among human rotaviruses. Majority of the isolates were of long pattern (66.67%), whereas short pattern was detected only in one third of the viruses. This preliminary study emphasizes for further detailed studies on the molecular characterization of rotaviruses circulating in this part of country and their relationship with other human rotavirus strains and animal strains in the country.

Keywords Diarrhea · detection · Group A human rotavirus · RNA-PAGE · electropherotypes

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Introduction

Diarrheal diseases are perpetually posing threat to global health status, particularly affecting vulnerable groups like young children of the developing nations. Rotavirus gastroenteritis among infants and young children remains a major cause of mortality in developing countries and a significant cause of morbidity in the developed countries¹. Rotaviruses are classified into groups A to F, in which group A rotavirus being known to exhibit the highest prevalence and pathogenicity. The virus has a genome of 11 segments of double-stranded RNA. Analysis of the electrophoretic mobilities of the 11 segments of dsRNA by PAGE yields a pattern, which is both constant and characteristic for a particular rotavirus isolate². Epidemiological and molecular studies in many countries show complex patterns of change year to year in the serotypes and electropherotypes that cause diarrhea in children from the same geographical areas³. Although relatively few studies have examined the molecular epidemiology of human rotaviruses in India^{4,5} report is still lacking from central India. Present study investigated the cause of gastroenteritis in hospitalized diarrheic children in this region of India and existence of genomic diversity amongst the circulating strains.

Materials and Methods

Samples collection and processing: During the study period, a total of 46 stool samples of children admitted to medical hospitals in Madhya Pradesh, central India (including 13 non-diarrheic samples from livestock farm workers' children) were collected and divided into different age groups viz. 0–1 year, 1–2 years, 2–3 years and more than 3 years of age. A volume of 10–20 ml of stool sample was collected in

sterile zip lock polythene bags, kept on ice and transported to the laboratory for immediate processing.

Stool suspension (10%) was prepared with phosphate buffer saline (PBS; pH 7.2) following centrifugation at 10,000 rpm for 30 min to remove coarse debris. One ml supernatant was used for viral nucleic acid extraction and rest was archived.

Extraction of viral RNA: Viral nucleic acid extraction was carried out as per the method described by Herring and his associates⁶. The pellet was suspended in 2x RNA sample buffer for RNA-PAGE analysis.

RNA-Polyacrylamide Gel Electrophoresis (RNA-PAGE): RNA-PAGE was carried out for the detection of human rotaviruses on the basis of number of genome segments and their typical electrophoretic migration pattern in PAGE. The extracted viral RNA pellet was dissolved in 2x RNA sample buffer and electrophoresed at 150 V till the dye came out of the gel using 1x Tris-Glycine buffer.

Staining of the gel: The gel was silver stained following the standard protocol⁷. The stained gel was photographed and stored in 10% ethanol.

Rotavirus reference strains

Rotavirus reference strains RH1 and RH2 belonging to group A human rotavirus propagated and maintained in the Department of Animal Biotechnology, CCSHAU, Hisar, Haryana were used for comparison. RH1 was representative of short electropherotype pattern, while RH2 of long pattern.

Results and Discussion

Being segmented genome virus, rotavirus genomic segments get separated into individual discrete bands during electrophoresis. Each rotavirus group (A to E) shows a typical migration pattern which helps in its identification, however, minor changes in the genome sequences alters the electrophoretic mobility of the segment. During the present study, all the rotavirus isolates from infants/children showed a typical migration of dsRNA segments in 4:2:3:2 pattern, which is indicative of Group A rotaviruses (Fig.1). Similar pattern of group A rotavirus have been reported from Paraguay⁸, Botswana⁹ and other places among humans.

Rotaviruses are further divided into two main electropherotypes as "long electropherotype" and "short electropherotype" based on the movement of their genomic

Fig. 1 Human rotavirus showing typical Group A rotavirus electropherotypic migration pattern (4:2:3:2) with short and long electropherotypes.

Fig. 2 Human rotaviruses detected in diarrheic stool samples by RNA-PAGE. Lanes indicate: H32, H36, H37, H39, H40, H42 strong positive; H15, H34, H35, H38 and H41 weak positive samples; RH1, RH2 reference human rotavirus strains of short and long electropherotypes.

segment 10 and 11¹⁰. Out of 12 rotavirus positive samples, 4 (33.33%) had shown short pattern, whereas 8 rotavirus isolates showed long pattern (66.67%) (Fig.2; Table3). Prevalence of long electropherotype rotavirus strain was more (66.67%) in comparison to short electropherotype (33.33%). Though, findings of present study are in accordance with earlier work reporting 80%¹¹, 75%¹², 84%⁴ prevalence of long electropherotypes in human children. Our findings are contrary to the report from Chennai, where short RNA pattern was seen in majority (75.4%) of the cas-

es¹³. During the present study, variations in the migration of individual segments were observed amongst the short electropherotypes as well long electropherotypes (Table 3).

Comparison of rotaviral RNA segments of field virus isolates revealed circulation of 5 different electropherotypes in this region (Table 3). Out of these 5 electropherotypes, 3 were of long pattern (A, B and C) and 2 were of short pattern (D and E). Electropherotype B was more prevalent with the incidence of 50% (Table 3). Six electropherotypes were reported to be circulating among children of Manipur¹⁴. In Chandigarh only 2 electropherotypes were found¹⁵ whereas 10 electropherotypes were reported in Bangalore¹⁶. Nine different electropherotypes were identified during

13 months study period in diarrheic children in Yogyakarta, Indonesia¹². This shows that number of electropherotypes varies in different areas due to genomic diversity, indicating a need for regular monitoring to develop a vaccine for the circulating electropherotypes.

Rotavirus infections in human beings are well documented with a variable degree of prevalence. In the present study, 36.36% (12/33) rotavirus infection was detected among diarrheic children in central India (Table 1). Similar prevalence (36%) in human children was reported in Bangalore¹⁶, whereas in a later study 21.6% prevalence was reported in Bangalore and only 11.2% in Mysore⁵. Lower prevalence (22.55%) of human rotavirus was also recorded among children with acute diarrhea in Chennai¹¹. Similarly in Vellore, only 18% prevalence was detected¹³. Prevalence of human rotavirus varies with places as in Manila, Philippines, 30.6%¹⁷ and in Jordan, 32.5% prevalence¹⁸.

In addition to 33 diarrheic fecal samples, 13 non-diarrheic children of farm-workers under the age of 5 years were screened for rotavirus infection, but none of them was found positive for asymptomatic rotavirus infection. This screening was intended to decipher the transmission of animal rotaviruses to human beings as the selected farms were found to be heavily positive for animal rota-

Table 1 Prevalence of rotavirus in diarrheic children.

Age (Years)	No. of cases	Positive for rotavirus	Percentage
0-1	22	09	40.91
1-2	06	00	00.00
2-3	01	01	100.00
3	04	02	50.00
Total	33	12	36.36

Table 2 Sex and age-wise prevalence of rotavirus in diarrheic children.

Age (Years)	No. of cases	Male		No. of cases	Female	
		Positive	Percentage		Positive	Percentage
0 – 1	13	04	30.77	09	05	05.56
1 – 2	04	00	00.00	02	00	00.00
2 – 3	01	01	100.00	00	00	00.00
≥3	02	01	50.00	02	01	50.00
Total	20	06	30.00	13	06	46.15

Table 3 Migration pattern and variability in electropherotypes detected in central India.

Electro-pherotype	Pattern	Type of Electro-pherotype	Variations in segments	No. of samples showing this pattern (Total 12)	Percent prevalence of electro-pherotypes
A	4:2:3:2	Long	Segment 2&3 distant; Segment 7,8&9 co-migrating	H15 (01)	08.33
B	4:2:3:2	Long	Segment 2&3 close; Segment 5&6 close; Segment 7&8 co-migrated or very close	H35, H36, H40, H41, H42, H46 (06)	50.00
C	4:2:3:2	Long	Segment 2&3 close; Segment 5&6 distant; Segment 7&8 co-migrating but segment 9 distant	H38 (01)	08.33
D	4:2:3:2	Short	Segment 7&8 co-migrating; Segment 10&11 close	H32, H34, H37 (03)	25.00
E	4:2:3:2	Short	Segment 7&8 co-migrating; Segment 10&11 distant	H39 (01)	08.33

virus. Results suggested that the susceptible age group for rotavirus infection was up to 1 year, as the incidence was 40.91%, in comparison to higher age groups.

Sex-wise incidence of rotavirus infection among male and female was 30% (6/20) and 46.15% (6/13), respectively. The result clearly indicated that female diarrheic children were more susceptible to rotaviral diarrhea than males. However, no association of rotavirus infection could be discerned between male and female child in a recent report in Chennai¹¹.

The results of present study clearly indicate that human rotavirus belonging to group A rotaviruses are circulating among infants/children of Madhya Pradesh, India. This emphasizes the need to further characterize the isolates at molecular level to make a candidate vaccine based on local strain of human rotavirus.

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