

Aetiology of childhood viral gastroenteritis in Lucknow, north India

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Background & objectives: Due to limited availability of data on viral aetiology of acute gastroenteritis in north India, the present study was planned to detect rotavirus, norovirus, sapovirus and astrovirus in stool samples of both in hospitalized and non-hospitalized children less than five years of age presenting with acute gastroenteritis.

Methods: A total of 278 stool samples from equal number of children were tested for rotavirus antigen using ELISA and for norovirus, sapovirus and astroviruses by reverse transcription (RT)-PCR.

Results: Of the 169 samples from hospitalized patients, rotavirus, norovirus, sapovirus and astrovirus were detected in 19.5, 2.3, 3.5 and 2.9 per cent samples, respectively. Of the 109 samples collected from the non-hospitalized patients, frequency of rotavirus and sapovirus detection was 9.1 and 1.8 per cent, respectively while norovirus and astrovirus were not detected.

Interpretation & conclusions: Rotavirus was the most frequent cause of viral gastroenteritis in both hospitalized and non-hospitalized children. Maximum positivity of the viruses was seen in children less than two years of age.

Key words Astrovirus - diarrhoea - norovirus - rotavirus - sapovirus - stool samples - viral gastroenteritis

Acute gastroenteritis is one of the most common childhood diseases, especially in developing countries. Annually 2.5 million deaths are estimated to occur due to enteric infections, greatly impacting children younger than five years of age¹. Over 20 different types of viruses have been identified as aetiological agents for gastroenteritis². Group A rotaviruses are considered to be the main agents followed by calicivirus, adenovirus and astrovirus³. In a cross-sectional study conducted at Lucknow, Uttar Pradesh, India, rotavirus was shown to

be associated with dehydrating diarrhoea, particularly in children 0-7 months of age⁴. Another study conducted in the same city⁵ also reported rotavirus as a major cause of acute diarrhoea in children aged two months to two years.

This study was performed to detect rotavirus, norovirus, sapovirus and astrovirus in stool samples of both hospitalized and non-hospitalized children, less than five years of age presenting with acute gastroenteritis in Lucknow, north India.

Material & Methods

This cross-sectional study was conducted in the Virology Laboratory, Post Graduate Department of Microbiology, King George's Medical University (KGMU), Lucknow, Uttar Pradesh, India, during August 2010 to July 2012. A total of 278 stool samples from the equal number of consecutive children less than five years of age, presenting with acute gastroenteritis were enrolled. A case of acute gastroenteritis was defined as a child less than five years of age presenting with watery diarrhoea of less than 14 days duration. Written informed consent was obtained from the parents and the study was approved by the ethics committee of the institution. Cases from either paediatric wards/emergency centre of the Gandhi Memorial and associated hospital or Shivgarh area in Lucknow district (non-hospitalized community patients screened through house-to-house survey) were enrolled. Patients presenting with bloody diarrhoea/with diarrhoea of more than 14 days/or parents declining consent were excluded. Relevant clinical data on age, sex, details of diarrhoea, presence of fever, vomiting and clinical signs and symptoms of dehydration were collected from either patient's records or from the patient's attendant.

Stool sample was collected as mentioned in CDC (Centers for Disease Control and Prevention) guidelines⁶. At least 10 ml of liquid, watery stool and one sample per patient was collected in a clean and dry screw capped, wide mouth plastic container. Samples from the hospital were transported to the laboratory immediately. The samples from the community were transported to the laboratory on the same day maintaining the cold chain.

Samples were processed without delay. ELISA for rotavirus group specific antigen (VP6) detection was done by a commercial kit (RIDASCREEN Rotavirus supplied by R-Biopharm AG, Darmstadt, Germany). One step reverse transcription (RT)-PCR assays for norovirus, sapovirus and astrovirus detection were performed using specific primers⁷⁻⁹. Stool suspension (10%) was prepared in 0.01 M phosphate-buffered saline (PBS) (pH 7.2), vortexed and centrifuged. The supernatant was transferred to a sterile, diethyl pyrocarbonate treated centrifuge tube. RNA extraction was done using a commercial kit (QIAamp viral RNA mini kit, QIAGEN, Gmbh Hilden, Germany) and the extracted RNA was subjected to reverse transcription to form cDNA using random hexamer primer (Amersham-Pharmacia Biotech, USA). Total

35 cycles of amplification were performed and results were analyzed by 1.5 per cent (w/v) agarose gel electrophoresis using molecular weight marker (MBD-13J 100bp DNA ladder, Bangalore, Genei, India) using 264 nm wavelength UV transilluminator and Gel Doc (Alphamager 3400 HP, Alpha Innotech, USA). A band of 329 bp for norovirus, 320 bp for sapovirus and 449 bp for astrovirus of amplified cDNA if visualized along with the band of positive control at the same base pair was considered positive. Positive, negative and extraction controls were run with each batch.

Statistical analysis: Chi-square test was applied to find the risk of viral positivity by age of the patients. The relative risk (RR) with its 95% confidence interval (CI) was calculated to find the risk of viral positivity by symptoms. The data analysis was carried out by using EPI INFO Windows version 7 (<http://www.cdc.gov/epiinfo>).

Results & Discussion

Of the total of 278 patients, 169 (60.8%) were enrolled from hospital and 109 (29.2%) from the community. Of the 278 stools samples, 54 (19.4%) were found positive for the tested viruses. Viral detection was more frequent in hospitalized (n=43, 25.4%) than in non-hospitalized (n=11, 10.1%) children (Table I). The relative risk of viral positivity by symptoms of fever, vomiting and dehydration is shown in Table II. Dehydration could not be properly assessed in non-hospitalized patients, hence, not shown in data.

The viral positivity was higher among hospitalized children less than two years of age (data on age not shown). Overall, the viruses were isolated during the cooler months of year with a peak in month between October and December among hospitalized patients (data not shown). Seasonal distribution among non-hospitalized patients could not be assessed due to small number of samples in each month.

Our study demonstrated that rotavirus was the leading cause of viral gastroenteritis with positivity rate of 16.6 per cent in hospitalized children and 8.3 per cent in non-hospitalized children followed by sapovirus (2.9%), astrovirus (1.8%), norovirus (1.2%) in hospitalized children. The rotavirus positivity in this study was comparable with the previous studies from Lucknow^{4,5}, although higher rates have been reported from Kolkata (48.1%)¹⁰ and Delhi (39.2%)¹¹. In developing countries norovirus is recognised as the second most frequent viral cause of childhood gastroenteritis after rotavirus¹². We found a low

Table I. Viral aetiology of gastroenteritis in patients from hospital and community

	Samples from hospitalized patients (n = 169) n (%)	Samples from non-hospitalized patients (n = 109) n (%)
Total positive	43 (25.4)	11 (10.1)
Rotavirus alone	28 (16.6)	9 (8.3)
Norovirus alone	2 (1.2)	0 (0.0)
Sapovirus alone	5 (2.9)	1 (0.9)
Astrovirus alone	3 (1.8)	0 (0.0)
Total mixed infection	5 (2.9)	1 (0.9)
Rotavirus with norovirus	2 (1.2)	-
Rotavirus with sapovirus	1 (0.7)	1 (0.9)
Rotavirus with astrovirus	2 (0.7)	-

Table II. Clinical features in children with virus positivity in patients.

Symptoms	Hospitalized patients (n=169)		Non-hospitalized patients (n=109)	
	Positivity for virus/total positive for symptom	RR (95% CI) <i>P</i> < 0.0001	Positivity for virus/total positive for symptom	RR (95% CI) <i>P</i> <0.0009
Fever	36/98 (36.7%)	3.73 (1.76-7.89) <i>P</i> < 0.0001	5/31 (16.1%)	1.80 (0.62-5.24)
Vomiting	37/73 (50.7%)	8.11 (3.62-18.18) <i>P</i> <0.0001	6/18 (33.3%)	5.06 (1.84-13.91) <i>P</i> <0.0009
Dehydration	39/102 (38.2%)	6.40 (2.40-17.09) <i>P</i> <0.0001	NA	NA

NA, not available

positivity of norovirus (1.2%), in hospitalized children compared with the other regions like Pune (8.6%), Maharashtra¹³ and Kolkata (4.8%)¹⁰. Sapovirus is known to cause symptomatic infection predominantly in infants and young children⁸. The detection rate of sapovirus reported from Vellore (5.1%) and Kolkata (2.3%) was similar to that found in our study (2.9%), while Delhi reported it to be 10.1 per cent in hospitalized patients^{10,14,15}. Prevalence of human astrovirus infection ranges from 0.3-10 per cent in children worldwide¹⁶. Its positivity in the present study (1.8%) among hospitalized patients was close to that reported from Pune (3.1%) and Kolkata (5.8%)^{17,18}. The conditions like season of sampling, socio-economic level of the population may explain differences in the detection rates.

As seen in our study fever, vomiting and dehydration are usually associated features of acute gastroenteritis more so of viral gastroenteritis¹⁹. The existence of co-infections makes the determination

of the primary aetiological agent, as well as the determination of virus specific symptoms difficult, however, the mixed infections were low in the present study and rotavirus was always the common detected pathogen.

Diarrhoea causing adenoviruses are known to be associated with gastroenteritis in children¹⁹. Diarrhoeal pathogens occur at a high frequency in developing countries thus, may be present in a notable proportion of control subjects as well as patients²⁰. Thus, inclusion of control in diarrhoeal studies play a major role, especially when comparing the relative contribution of different pathogens to the overall burden of disease.

In conclusion, rotavirus was the major viral pathogen in both hospitalized and non-hospitalized children with acute gastroenteritis. Other viruses like norovirus, sapovirus and astrovirus were found to be associated with acute gastroenteritis in a small number of hospitalized children.

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