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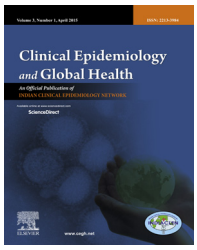

Original Article

Rotavirus associated acute gastroenteritis among under-five children admitted in two secondary care hospitals in southern Karnataka, India

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ABSTRACT

Background: Rotavirus infection is the common cause of severe diarrhea among under-five children. Globally it is responsible for majority of the hospital visits and admissions due to diarrhea in this age group. This hospital-based surveillance aimed to assess the burden of rotavirus diarrhea and to identify its prevalent strains among under-five children.

Methods: The study was conducted in two secondary level health care facilities of Manipal University, Manipal during November 2011 through July 2012. All under-five children admitted with acute diarrhea were recruited into the study.

Results: A total of 95 children were admitted with acute diarrhea during the study period. Of the 95 stool samples collected, 14 were inadequate and 81 samples were tested for the presence of rotavirus using commercial enzyme immunoassay kit (Premier Rotaclone Qualitative ELISA). Rotavirus positive samples were shipped to Central laboratory at CMC, Vellore for strain surveillance and characterization. Out of the 81 stool samples tested for rotavirus, 31 samples (38.3%) were positive for rotavirus VP6 antigen. Rota positivity was observed to be highest during the month of December (29.0%) and lowest in the month of June. Majority of the rotavirus positive cases (45.2%) were among children aged 13–24 months and among those who had very severe diarrhea (56.5%). The most common genotypes identified were G1P[8] and G2P[4] strains (25.8% each).

Conclusions: Though the burden of overall diarrhea among under-five children is not very high in this area, the proportion of rotavirus diarrhea among the hospitalized children is considerably high.

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1. Introduction

Acute diarrheal diseases (ADD) is one of the major causes of death among under-five year children in India, accounting for 13% of mortality (300,000 deaths annually) in this age group.[1,2] About 25–50% of these cases are due to rotavirus infection leading to a large number of outpatient visits, hospitalizations, and deaths among under-five year children.[3,4]

Globally, the prevalence of rotavirus diarrhea among under-five children ranges from 6% to 56%, whereas the incidence of rotavirus diarrhea reported in India varies from 5% to 70%.[2,4–6] No seasonal variation in rotavirus diarrhea has been reported in tropical areas, though the frequency is observed to be higher during the initial months of the year.[7] As per the evidence, initial infection with rotavirus offers immunity to an extent due that subsequent rota infections tend to be less severe.[8] Rotavirus infection is found to be the commonest cause of diarrhea with severe dehydration which requires hospitalization among children in India and worldwide.[5,9] In India, stool examinations are normally not done to isolate the organisms during acute diarrheal episodes. Typically the children with rotavirus infection present with triad of symptoms, fever, projectile vomiting, and profuse watery diarrhea, which may lead to moderate to severe dehydration in the infected child. But due to the high cost involved, these cases are rarely diagnosed and are treated symptomatically with oral rehydration salts (ORS) solution or intravenous (IV) fluids.

Hence, this study was undertaken with an objective to carry out a hospital-based surveillance of rotavirus gastroenteritis among under-five children to estimate the disease burden and to identify the prevalent strains of rotavirus in the population under surveillance in southern Karnataka, India.

2. Materials and methods

The present study is a part of a larger multi centric prospective hospital based surveillance study.[10] At our site, the study was conducted from November 2011 to July 2012 in a private health care set up that included two secondary health care facilities attached to a tertiary care hospital which serves as a referral center. These health care facilities located at the taluka level provide services to both urban and rural population of the local area.

As per the study protocol, all children below 5 years of age, who presented with acute diarrhea (defined as ≥3 looser than normal stools with or without vomiting during the preceding 24 h period) to these health facilities and required hospitalization and rehydration for at least six hours were considered eligible for the study. These children were recruited in the study following a written informed consent obtained from the parents. A diarrheal hospitalization logbook was used to count and track all the eligible children recruited into the study.

The study was time bound and all eligible and consenting children during the study period were included in the study.

2.1. Clinical assessment of the subjects

The clinical parameters such as duration and maximum number of episodes of diarrhea and vomiting, intensity of fever and dehydration were considered to assess the severity of diarrheal episodes among the recruited children. These parameters were recorded in a pre-tested case report form. Severity of diarrhea was graded using the Vesikari scoring system.[11] As per the grading, a child with a score between 0 and 5 was considered to have mild diarrhea, 6 and 10 moderate, 11 and 15 severe and a score of ≥16 was considered to be having very severe diarrhea.

2.2. Stool specimen collection

A single stool sample (approximately 5 ml) was collected in a labeled sterile screw-top container from the consenting subjects either on the day of admission or within 48 h of hospital admission. The container was checked for labeling and leakage, immediately transported in a vaccine carrier to the laboratory at Centre for Virus Research located at the study site and stored in a deep freezer at −20 °C until the relevant testing was done. Care was taken to avoid freeze–thaw cycles for the stool specimens.

2.3. Detection of rotavirus

The stool samples were tested for rotavirus VP6 antigen using a commercial Enzyme Immuno Assay (EIA) kit (Premier Rota clone Qualitative EIA, Meridian Bioscience Inc., Cincinnati, USA) in duplicates and with appropriate controls. All the rotavirus VP6 antigen positive stool samples were shipped to the Central laboratory at Department of Gastrointestinal Sciences, Christian Medical College, Vellore under appropriate controlled conditions for strain surveillance and characterization.

The study was conducted as per the Code of Ethics of the World Medical Association (Declaration of Helsinki), GCP guidelines issued by the Central Drug Standards and Control Organisation, India and the ethical guidelines by Indian Council of Medical Research. The study protocol was approved by the Institutional Ethics Committee prior to the initiation of the study. The study was formally registered under the Clinical Trials Registry – India with a registration number of CTRI/2012/03/002475.

2.4. Data analysis

The data from case report forms, diarrheal log book and genotype reports was extracted into SPSS (Statistical Package for Social Sciences) version 16.0 and analyzed. The results are expressed as frequencies and proportions. Chi-square test was applied wherever applicable and a p-value of <0.05 was considered as statistically significant.

3. Results

A total of 95 under-five year children hospitalized with acute diarrhea were recruited in the study. As is observed from

Table 1 – Distribution of overall diarrhea & rotavirus positive diarrhea among the study population.

Characteristics	Total recruited children ($n = 95$) No. (%)	Rotavirus infected children ($n = 31$) No. (%)
Age group (in months)		
0–6	6 (6.3)	0 (0)
7–12	24 (25.3)	6 (25.0)
13–24	33 (34.7)	14 (42.4)
25–59	32(33.7)	11(34.4)
Gender		
Male	59 (62.1)	19 (32.2)
Female	36 (37.9)	12 (33.3)
Severity of diarrhea (Vesikari grading)		
Mild	0	0
Moderate	12 (12.6)	1 (8.3)
Severe	58 (61.1)	17 (29.3)
Very severe	25 (26.3)	13 (52.0)

Table 1, proportion of rotavirus infected children was found to be higher among the children aged 13–24 months as compared to those aged ≤12 months and >24 months. No gender wise difference was observed for rotavirus positivity. It increased with the severity of diarrhea with more than half of the very severe diarrhea cases being rotavirus positive.

Of the 95 children recruited into the study, stool samples could be collected from 88 children within 48 h of hospitalization of which 81 samples were analyzed. Seven samples were inadequate and could not be analyzed. Of the 81 stool samples, 31 (38.3%) were found to be positive for rotavirus.

As depicted in Table 2, a higher proportion (93.5%) of the rotavirus positive children had vomiting associated with loose stools compared to rota negative children (61.2%) and the difference was found to be statistically significant ($p < 0.05$). The mean Vesikari scoring that provides an indication of the severity of diarrhea was found to be higher for the rotavirus positive group.

On comparing rotavirus with non-rotavirus diarrhea, no statistically significant association was observed between age group and rotavirus ($p = 0.13$) or with gender and rotavirus ($p = 0.94$).

Table 2 – Characteristics of rotavirus-infected and uninfected children hospitalized with acute gastroenteritis.

Characteristics	Rotavirus infected children ($n = 31$)	Rotavirus uninfected children ($n = 50$)
Mean age in months ± SD	23.3 ± 13.3	20.3 ± 13.7
Mean duration of hospital stay ± SD	4.2 ± 1.3	4.1 ± 2.1
Associated with vomiting*	29 (93.5%)	30 (61.2%)
Max. no. of episodes of vomiting in 24 h (for >5 times)	12 (38.7%)	9 (18.0%)
Diarrhea (for >3 days)	20 (64.5%)	29 (58.0%)
Max. no. of episodes of diarrhea in 24 h (for >5 times)	18 (58.1%)	29 (58.0%)
Mean Vesikari score, ±SD	14.9 ± 3.1	12.7 ± 2.7

* $p < 0.05$.

Table 3 – Rotavirus positivity across the category of diarrhea severity among the study population based on Vesikari Scoring system.

Severity of diarrhea	Rotavirus status in the stool samples		Total
	Positive No. (%)	Negative No. (%)	
Moderate	1 (9.1)	10 (90.9)	11
Severe	17 (36.1)	30 (63.8)	47
Very severe	13 (56.5)	10 (43.5)	23

On assessment of severity of diarrhea using Vesikari scoring system, 61.1% of the children were found to have severe diarrhea followed by very severe (26.3%) and moderate diarrhea (12.5%) as shown in Table 3. The category of moderate and the severe diarrhea had a larger proportion of rota negatives, whereas the proportion of rota positives was almost twice as compared to the rota negatives among those, who had very severe diarrhea. However, no significant association was found between the severity of the cases and rotavirus positivity ($p = 0.14$).

As depicted in Fig. 1, a distinct peak was observed during the month of December for all diarrhea cases as well as rota positive cases. The number of cases for the months of August, September and October 2012 could not be estimated as the study was initiated in November 2011 and was completed by July 2012.

Of the total recruited children, 77 (81.0%) were managed with IV fluids followed by oral rehydration therapy (ORT) and the rest (18.9%) required only ORT. There was no mortality reported in the study.

Strain characterization was performed for all VP6 positive stool samples. Genotyping was performed at the Central Laboratory using reverse-transcription polymerase chain reaction (RT-PCR). Commonest G type was G1 and G2 and commonest P type was P[4] and P[8]. Of the 31 samples tested positive for rotavirus, 8 samples (25.8%) each had G1P[8] and G2P[4] strains, followed by G2P[6] in 2 samples (6.5%) and G2P [8] and G9P[8] strains in one sample each (3.2%) as shown in Fig. 2. Combination of strains such as G1P[4,8], G1P[6,8], and G1G2P[4,8] were detected in 7 samples (22.6%).

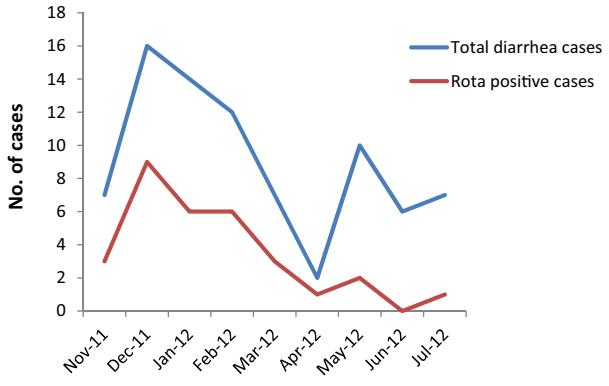

Fig. 1 – Month wise distribution of diarrhea cases ($n = 95$).

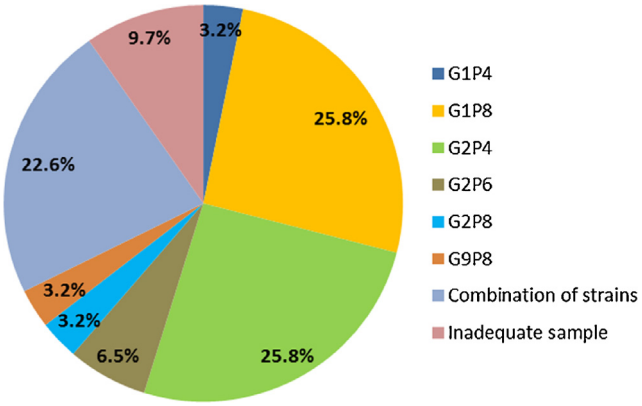

Fig. 2 – Distribution of rotavirus strains as per G & P typing ($n = 31$).

4. Discussion

This was a hospital based surveillance study and we had relatively a smaller sample, hence the results may not give the true picture of the disease burden in the community. The reason for getting a small sample would be the low prevalence of ADD among under-five children in this region (4.2%) compared to an overall prevalence of 9% as per national statistics.[1,12,13] This may be attributed to the factors such as high literacy rate, better socio economic status and a good awareness level regarding hygienic practices among the population in this part of Karnataka.

We aimed to estimate the burden of rotavirus gastroenteritis in children less than five years of age admitted with diarrhea through a hospital based surveillance. The overall rotavirus positivity rate was found to be 38.3% in our study, which is within the range of 21–50% as reported by various Indian studies.[14–22]

In the present study the maximum (34.7%) proportion of gastroenteritis cases were from 13 to 24 months age group and it was observed that the same age group had a higher proportion (45.2%) of rotavirus positive cases. This is in contrast to the findings from various studies which reported a higher proportion of diarrhea cases (43–87%) among children below 12 months of age.[14,15,17] Also they found that most of the rotavirus positive cases were in the age groups of 24–35 months (46.9%),[14] 37–48 months (58.5%)[15] and 1–12 months (68.7%)[17] as against to our findings. In the present study, though we had 6 diarrhea cases (6.3%) from below 6 months of age, none of them were positive for rotavirus.

Various studies carried out across India have reported peaks in rotavirus diarrhea cases during the spring and winter season (59–72%) of the year, which is in agreement with our study findings in which we observed a substantial number of rota positive cases (49.8%) occurring during the winter months ($p < 0.05$).[23–27] This is in contrast to the general understanding that such peaks are usually not observed in southern India which has a more tropical climate.[6,19,28]

Tracking the rotavirus strains in a particular region is important to understand the epidemiology of the disease and also helps in identifying the changes following the introduction of a vaccine.

As there is paucity of data in this regard from Karnataka, this study is first of its kind, which is intended to identify the distribution of various rotavirus strains in this region. The present study provides information on both rotavirus G and P types. Based on our findings, G1P[8] and G2P[4] were the most prevalent strains (25.8% of the cases in each group) among under-five children in this area. This is comparable with the finding of another study conducted in Vellore, which showed that G1P[8] (44.6%) and G2P[4] (18.4%) were the commonly detected rotavirus strains among under-five children having diarrhea.[22] Similarly, a review done by Ramani and Kang et al. reported that G1P[8] was the most common (27%) rotavirus strain identified in various parts of India.[18] A multicentric study conducted in different regions of India by the national Indian surveillance network found that the most commonly observed G and P type combinations were G2P[4] and G1P[8] (25.7% and 22.1% respectively), which is consistent with our study findings.[20] Previous studies done by Mathew et al. and Badur et al. have also observed G1P[8] to be the most common strain with a very high prevalence (49.7% and 56.2% respectively).[14,17] Our finding is also supported by a review conducted by Kahn et al. indicating that G1P[8] (35.0%) and G2P[4] (21.0%) were the commonest rotavirus strains identified in various studies from northern as well as southern India.

Studies conducted in different parts of India have observed a higher prevalence of various combinations of G9 and G12 strains, but these were either not found or very few in numbers in the present study.[14,17,19]

In conclusion, it is evident from the study that though the prevalence of diarrhea among under-five is comparatively low in the study area, a substantial proportion of diarrheal cases reporting to the health facilities are due to rotavirus infection which emphasizes the need for early vaccination against rotavirus. This study also highlights the most common circulating rotavirus strains in this area and the need for continuous monitoring of strains evolution.

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Conflicts of interest

One of the authors is employed by Shantha Biotechnics Ltd., Hyderabad, India and was involved in planning of the study and in manuscript editing. All other authors are faculties of Manipal University, Manipal.

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