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Rotavirus gastroenteritis among children less than 5 years of age in private outpatient setting in urban India☆

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ABSTRACT

Burden of rotavirus gastroenteritis (RVGE) in outpatient setting in India is not fully understood. A prospective study was undertaken to describe RVGE among Indian children less than 5 years of age presenting in outpatient departments with acute gastroenteritis (AGE). This study was conducted at 11 outpatient departments (OPDs) of private pediatric clinics in urban areas of India. A total of 605 eligible children were enrolled at OPDs. Stool samples of the subjects were collected and tested for presence of rotavirus antigen by enzyme immune assay (EIA) and were typed by reverse-transcriptase polymerase chain reaction (RT-PCR). Physician examined the children and documented the disease particulars. In addition, parents/guardians were interviewed for AGE related symptoms, health care utilization and cost incurred due to AGE, and parental stress associated with AGE. After OPD, parents/guardians completed diary cards and questionnaires to capture the information for 14 days following the enrollment.

Complete data for analysis including stool sample results was available from 552 subjects. 23% (127/552; [CI 19.5, 26.5]) of stool samples were rotavirus (RV) positive. RT-PCR was done for 85.8% (109/127) of RV positive samples. G1, G2, G9, and G12 types were identified in 34.9% (38/109), 37.6% (41/109), 8.3% (9/109), and 6.4% (7/109) stool samples, respectively. P[4] and P[8] were identified in 36.7% (40/109) stool samples each, followed by P[6] identified in 15.6% (17/109) stool samples.

At the time of enrollment, all three symptoms (vomiting, diarrhea, and fever) were observed concurrently in higher proportion of RV positive subjects compared to RV negative subjects (60.6% [77/127] vs. 42.8% [182/425], $p = 0.0004$). Healthcare resource utilization, costs incurred due to disease, and parental stress were higher for RV positive subjects compared to RV negative subjects.

In conclusion, RVGE was found to be a definite burden in AGE cases attending pediatric outpatient clinics in urban areas and it was associated with substantial economic and psychological burden. Introduction of rotavirus vaccine in India may help in reducing this disease burden.

1. Introduction

The burden of diarrhea caused by rotavirus infection in the pediatric population is a major cause of concern worldwide. It is estimated that in 2008, rotavirus diarrhea or rotavirus gastroenteritis (RVGE) resulted in 453,000 deaths worldwide in children aged less than 5 years, which accounted for 5% of all deaths in this age group [1]. This estimate is after excluding the post vaccine

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introduction data if any. India alone accounted for approximately 22% of world RVGE deaths (98,621 deaths) in children aged less than 5 years [1]. These figures clearly indicate high burden of rotavirus mortality among Indian children. Rotavirus associated morbidity in India is also well documented. Many Indian studies including the Indian Rotavirus Strain Surveillance Network (IRSN) have evaluated RVGE burden amongst hospitalized cases of acute gastroenteritis (AGE) and some studies also demonstrated rotaviruses strain diversity as in other developing countries [2–6]. These hospital based studies included testing stool samples for rotavirus and to determine the causative rotavirus strains. However, well designed study data is not available with respect to burden of RVGE as well as causative rotavirus strains when AGE cases are enrolled in pediatric outpatient settings and are followed up for the disease spectrum. We conducted an observational study to understand the epidemiological profile of RVGE in private outpatient settings in India. Earlier reports of studies conducted in hospitalized settings probably represent severe cases of RVGE that needed hospitalization, while the present study aimed to include information on disease caused by RVGE which is seen first in the outpatient department (OPD). The objective of the study was to describe RVGE in children aged less than 5 years who attended OPDs of private pediatric clinics in urban areas. Accordingly stool samples of AGE subjects were tested to determine rotavirus positivity and RV positive samples were tested for G and P types. Other characteristics of RVGE like clinical presentation, severity, economical and psychological impact on the parents/family of the children were also studied and compared to non-RVGE.

2. Materials and methods

2.1. Study design

This was an observational, prospective study conducted at 11 sites located in urban areas across all five geographical (north, south, east, west, and central) regions of the country. Children less than 5 years of age who attended the OPD of private pediatric clinics for the treatment of AGE were enrolled. The study was conducted over a period of 11 months (15 December 2011–14 November 2012); however individual sites differed in their study duration due to variation in AGE burden and monthly enrollment rate.

2.2. Study population

Parents/guardians of children aged less than 5 years (60 months) who suffered from AGE and attended OPD, were informed about the study in detail. Children who met the eligibility criteria were included in the study after written informed consent obtained from the parents/guardians. AGE was defined as three or more loose or watery stools and/or one or more episodes of forceful vomiting in a 24-h period. These symptoms must have occurred within 3 days prior to the OPD visit. Children who were enrolled in any other trial, or had history of rotavirus infection, or had received a rotavirus vaccine were excluded. Children with any such condition that in the opinion of the investigator could interfere with the study objectives were excluded. Children whose parents were unable to give consent were also excluded.

2.3. Data collection

After receiving written informed consent, the following information was gathered from the parent/guardian using questionnaire: subject's demographics including medical history, socio-economic details (e.g. annual family income, area of residence), and family details (e.g. number of members in family, number of siblings); information about direct costs (e.g. OPD, medicines, extra drinking fluids, expenses on conveyance for visit), and impact caused by RVGE (e.g. monetary impact of lost days of work for parent/guardian and parental stress). The monetary impact of lost days of work was calculated based on daily wages of the parent/guardian. The stress suffered by the parent/guardian due to child's disease was scored on a scale of 0–10, where '0' was no stress and '10' was extreme stress.

At enrollment, following detailed clinical data were recorded using questionnaire: date of onset of symptoms (diarrhea, vomiting, and fever), number of days for which each symptom continued, maximum frequency of stools and vomiting episodes per day, maximum temperature recorded, dehydration status, behavioral signs and symptoms, and treatment given to the subject. The severity of dehydration of the subject was assessed as mild, moderate, or severe by the investigator based on patient examination for restlessness, lethargy, sunken eyes, skin pinch, normal or poor feeding. The number of IV rehydration bottles administered to the subject was also recorded. Occurrences of behavioral signs and symptoms such as irritable/less playful, lethargic/listless, and convulsions were also recorded. The parent/guardian was given a diary card and questionnaires to record follow-up information on daily symptoms of the subject, and costs and impact caused due to the disease. The questionnaire used on the day of enrollment and follow-up questionnaires used to collect information after OPD visit or Day 1 were designed specifically for this study, and contained simple and easily understandable questions in local vernacular language. The parent/guardian was trained to fill the diary card and questionnaires.

Study personnel made two telephonic contacts with the parent/guardian, first after Day 7 and second after Day 14, for collecting follow-up information for Day 1–Day 7 and Day 8–Day 14, respectively. Additional information such as healthcare utilization (e.g. repeat OPD visit/s, hospitalization, intravenous [IV] hydration) and impact of disease and its progress during Day 1–Day 7 and Day 8–Day 14 was also collected telephonically.

2.4. Assessment of severity

The severity of AGE was scored by the physician based on physical examination of child and the information collected for the duration and severity of disease symptoms. Two scales, namely: Clark scale and Vesikari scale which have been used earlier in clinical trials on rotavirus vaccines were used. The Clark scale is a 24-point scale based on duration and frequency of diarrhea and vomiting, degree and duration of fever measured by rectal temperature, and description and duration of behavioral symptoms. Axillary temperature measurements were used instead of rectal measurements. Conversion of axillary temperature to rectal temperature was performed using following formula [7]: rectal temperature (°C) = 0.98 × axillary temperature (°C) + 0.8 (°C).

The Clark scale is divided into three ranges: mild <9, moderate 9–16, and severe >16.

The Vesikari scale is a 20-point scale based on duration and peak frequency of diarrhea and vomiting, degree of temperature, severity of dehydration, and treatment provided to the patient (i.e., rehydration or hospitalization). This scale is divided into three ranges: mild <7, moderate 7–10, and severe ≥11 [8,9].

2.5. Stool sample collection and laboratory analysis

Stool sample (1.5–5 g) was collected for each subject, preferably at enrollment, or later but within 14 days of the onset of AGE symptoms. The stool samples were stored at 2–8 °C. Samples were shipped to The Wellcome Trust Research Laboratory (Department of Gastrointestinal Sciences, Christian Medical College, Vellore, Tamil Nadu), which was the central laboratory for

this study. The samples were shipped in batches and laboratory testing occurred after the 14 days follows up of individual subject was over. Thus, the investigators or the site staff was not aware if subject was suffering from RVGE or non-RVGE when AGE related data was collected and severity scoring was done.

Stool samples were first tested for the presence of rotavirus antigen by enzyme immune assay (EIA) using Prospect™ Rotavirus EIA. The samples that were positive by EIA were genotyped for their respective G and P types by RT-PCR.

For RT-PCR, viral DNA was extracted from stool specimens and reverse transcribed using random primers to generate complementary DNA (cDNA). The cDNA was used as a template for genotyping in hemi-nested multiplex PCRs for VP7 and VP4 genes using published primers and protocols [10–14]. The primers could amplify VP7 genotypes: G1, G2, G3, G4, G8, G9, G10, and G12; and VP4 genotypes: P[4], P[6], P[8], P[9], P[10], and P[11].

2.6. Ethical conduct

The study was conducted in accordance with the ethical principles enshrined in the Declaration of Helsinki, International Conference on Harmonization (ICH) – Guideline for Good Clinical Practice (GCP), and all applicable local regulatory requirements. The study protocol was approved by the Ethics Committees for respective sites.

2.7. Data analysis

Per protocol (PP) population was used to analyze the study data. Subjects who had a total data of 14 days, EIA results available, and completed the study as per protocol were included in the PP population. The proportion of RVGE among AGE was calculated for regions and overall (with 95% CI). Data were summarized using number and percentages, mean, median and other statistics as appropriate. Continuous and categorical variables were compared across RV positive and RV negative subjects using Mann–Whitney U test and Chi-square test, respectively. A p-value <0.05 was considered as statistically significant. All statistical analysis was performed by SAS software version 9.1.3 (SAS Institute, Cary, NC, USA).

For planning the study, we assumed that approximately 20% of enrolled AGE cases might get detected as RV positive and it was based on earlier outpatient setting studies in India [15,16]. With this expected proportion of rotavirus positivity, 500 was the targeted (PP) population for enrolling AGE subjects. It was planned that each region would provide complete data of at least 100 subjects.

3. Results

We initiated the study at a total of 10 sites with two sites located in each geographical region (i.e., north, south, east, west, and center) of India. These sites started enrolling subjects from December 2011. Due to inadequate enrollment from one site and region as a whole, in northern region, we initiated enrollment at an additional site in July 2012, taking the total number of sites to 11. Enrollment at the new site and existing site in the region was competitive.

3.1. Study population

We screened a total of 616 children for eligibility for participation in this study (Fig. 1). We found 98.2% (605/616) eligible subjects and enrolled them in the study. The study collected stool samples from all subjects (n = 605). Site staff contacted the parent/guardian of all subjects (n = 605) by telephone for data collection after Day 7 and Day 14, for collecting information for Day 1–Day 7 and Day 8–Day 14, respectively. Out of 552 subjects in PP population, three sites in north India had 109 (16 + 59 + 34) subjects; two sites each in south, east, west, and center of India had 99 (47 + 52), 113 (55 + 58), 111 (58 + 53), 120 (45 + 75) subjects, respectively.

From majority of the subjects (89.7% [495/552]) stool samples were collected within 2 days of enrollment. EIA testing was possible for samples of 91.2% (552/605) subjects' stool samples. EIA testing could not be performed for stool samples of 8.8% (53/605) subjects for reasons such as insufficient quantity of samples (n = 46), samples not labeled (n = 4), and inappropriate enrollment (disease symptoms occurred >3 days prior to enrollment as opposed to protocol requirement) (n = 3).

In addition to laboratory results (for EIA), complete per protocol data was available for these 552 subjects and they formed the PP population.

3.2. Demographic characteristics

The demographic characteristics are presented in Table 1. Mean (±SD) age of subjects was 17.0 (±12.6) months. RV positive subjects were younger compared to RV negative subjects (mean age ± SD was 14.8 ± 10.1 months vs. 17.6 ± 13.2 months); this difference was not statistically significant. The distribution of cases by age revealed statistically significant (p = 0.0081) proportion of RV positive cases in ≤24 months age group. Other baseline characteristics including gender, ethnicity, family details and income were similar between RV positive and negative subjects (Table 1).

3.3. Burden of RVGE

Of stool samples of 552 subjects, 23.0% (127/552; [CI 19.5, 26.5]) were found RV positive. Rotavirus positivity was higher in the months of January (36.5% [19/52]), February (33.9% [19/56]), and March (38.7% [36/93]) (Fig. 2). Monthwise enrollment and rotavirus positivity for total PP population and region-wise is depicted in Fig. 2.

3.4. Strain characterization

RT-PCR was done for 85.8% (109/127) of RV positive samples (Fig. 3); for the rest of the samples, RT-PCR could not be done because of inadequate stool quantity. Among these 109 samples, we identified G1, G2, G9, and G12 in 34.9% (38/109), 37.6% (41/109), 8.3% (9/109), and 6.4% (7/109) stool samples, respectively. We identified P[4] and P[8] in 36.7% (40/109) stool samples each, followed by P[6] identified in 15.6% (17/109) stool samples. Most common GP types were G1P[8] and G2P[4] identified in 32.1% (35/109) and 27.5% (30/109) stool samples respectively.

We found mixed infection of more than one G type in 6.4% (7/109) stool samples which were all G1 + G2 type. Mixed P type infection was found in 4.6% (5/109) stool samples, which were P[4] + P[6], P[4] + P[8], and P[8] + P[6] in 1.8% (2/109), 1.8% (2/109), and 0.9% (1/109) stool samples respectively.

There were also some untypeable strains (G untypeable: 6.4% [7/109], P untypeable: 6.4% [7/109], and both G and P untypeable: 4.6% [5/109]).

3.5. Clinical data and severity scores

Table 2 describes the presence and duration of AGE symptoms during the study period. At enrollment, we observed the co-occurrence of all three symptoms (vomiting, diarrhea, and fever) in higher proportion of RV positive subjects compared to RV negative subjects (60.6% [77/127] vs. 42.8% [182/425], p = 0.0004). A higher proportion of RV negative subjects presented with only diarrhea (without vomiting and fever) compared to RV positive subjects (22.8% [97/425] vs. 10.2% [13/127], p = 0.0018).

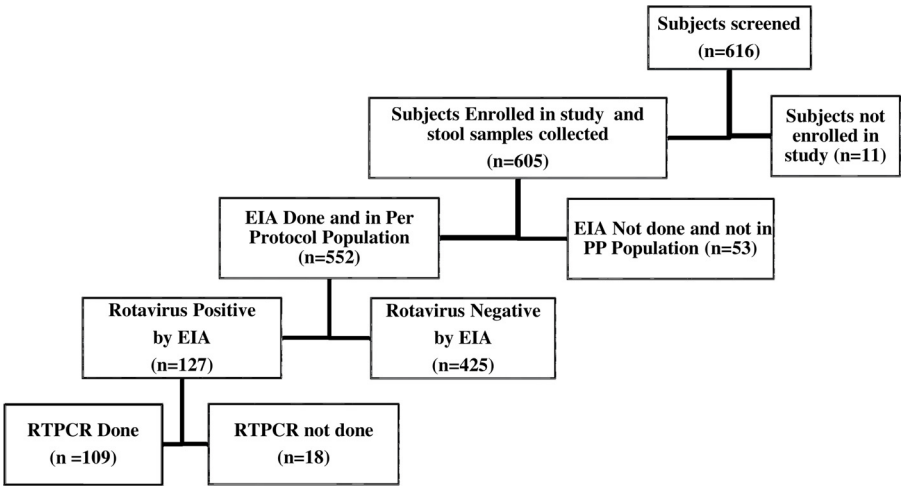

Fig. 1. Subject disposition flow chart.

The severity of RV positive and negative cases determined by Clark scale and Vesikari scale is presented in Table 2. The proportion of subjects with higher AGE severity was statistically significant among RV positive subjects compared to RV negative subjects by both the scales (Vesikari scale: $p = 0.0026$, Clark scale: $p = 0.0004$). For RV positive subjects, the disease was mild, moderate, and severe for 4.7% (6/127), 18.1% (23/127), and 77.2% (98/127) subjects, respectively by the Vesikari scale. By the Clark scale, disease severity was mild, moderate, and severe for 26.8% (34/127), 69.3% (88/127), and 3.9% (5/127) subjects, respectively.

3.6. Healthcare costs due to RV infection

The total direct cost including costs incurred prior to OPD visit, on the day of OPD visit, and from OPD till Day 14 were statistically higher ($p < 0.0001$) for RV positive subjects (3177 INR) compared with RV negative subjects (1787 INR). The total direct cost incurred for most subjects, i.e., 97.6% (124/127) RV positive and 98.6% (419/425) RV negative subjects was 10,000 INR or less. The details of direct costs incurred prior to OPD visit, on the day of OPD visit, and subsequent to OPD visit are presented in Table 3. The total direct cost was higher for subjects who were subsequently hospitalized (38 RV positive and 50 RV negative) compared to those who did not require hospitalization. The mean total direct cost for hospitalized subjects was 7158 INR and 6895 INR for RV positive and RV negative subjects, respectively. OPD treated subjects had significantly higher ($p < 0.0001$) mean total direct cost in RVGE positive subjects (1478 INR) as compared to RV negative subjects (1106 INR).

3.7. Healthcare resource utilization

Almost similar proportions of RV positive (14.2% [18/127]) and RV negative subjects (11.1% [47/425]) revisited the outpatient facility at least once after enrollment. Overall, a higher proportion

Table 1
Baseline characteristics of children presenting with AGE.

Characteristics	RV positive ($N = 127$), n (%)[a]	RV negative ($N = 425$), n (%)[a]	p-value
Age (mean ± SD) (months)	14.8 ± 10.12	17.6 ± 13.2	0.1312[b]
Age (months)			0.0081[c]
≤24	109 (85.8)	317 (74.6)	
>24–≤60	18 (14.2)	108 (25.4)	
Gender			0.2298[c]
Male	84 (66.1)	256 (60.2)	
Female	43 (33.9)	169 (39.8)	
Ethnicity (Indian)	127 (100.0)	425 (100.0)	
Children being breastfed at enrollment	83 (65.4)	242 (56.9)	0.0909[c]
Family type			0.6306[c]
Nuclear	86 (67.7)	278 (65.4)	
Joint	41 (32.3)	147 (34.6)	
Total members in the family			
Median	4	4	0.4481[b]
Family income (mean ± SD) (INR)	321,420.5 ± 349,763.57	345,291.6 ± 69,5417.78	0.8369[b]
Classification of family income (INR)			0.2536[c]
≤1,00,000	23 (18.1)	49 (11.5)	
1,00,001–5,00,000	87 (68.5)	318 (74.8)	
5,00,001–10,00,000	15 (11.8)	48 (11.3)	
>10,00,000	2 (1.6)	10 (2.4)	
Area of residence			0.0357[c]
Urban	116 (91.3)	408 (96.0)	
Rural	11 (8.7)	17 (4.0)	

For calculating percentage, denominator was the number of subjects in each group (127 in RV positive group and 425 in RV negative group)

[a] Applicable unless otherwise indicated.

[b] p-value calculated using Mann–Whitney U test.

[c] p-value calculated using Chi-square test.

Table 2
Clinical presentation and severity of AGE.

	Statistics	RV positive ($N = 127$)	RV negative ($N = 425$)	p-value
Symptoms presented at enrollment				
Only diarrhea	n (%)	13 (10.2)	97 (22.8)	0.0018[a]
Vomiting and diarrhea, without fever	n (%)	23 (18.1)	104 (24.5)	0.1351[a]
Diarrhea and fever, without vomiting	n (%)	14 (11.0)	42 (9.9)	0.7086[a]
Vomiting, diarrhea, and fever	n (%)	77 (60.6)	182 (42.8)	0.0004[a]
Symptoms and duration (in days) experienced over the course of disease				
Vomiting	n (%)	90 (70.9)	259 (60.9)	0.0418[a]
	Median (days)	2	2	0.0493[b]
	Range (min, max) (days)	1, 5	1, 6	
Diarrhea	n (%)	127 (100)	425 (100)	
	Median (days)	4	4	0.3080[b]
	Range (min, max) (days)	2, 11	2, 13	
Fever	n (%)	78 (61.4)	187 (44.0)	0.0006[a]
	Median (days)	2	2	0.1108[b]
	Range (min, max) (days)	1, 6	1, 7	
Abnormal behavior	n (%)	119 (93.7)	357 (84.0)	0.0054[a]
	Median (days)	3	3	0.6444[b]
	Range (min, max) (days)	1, 7	1, 10	
Vesikari scale				0.0026[a]
Mild	n (%)	6 (4.7)	31 (7.3)	
Moderate	n (%)	23 (18.1)	137 (32.2)	
Severe	n (%)	98 (77.2)	257 (60.5)	
Clark scale				0.0004[a]
Mild	n (%)	34 (26.8)	193 (45.4)	
Moderate	n (%)	88 (69.3)	226 (53.2)	
Severe	n (%)	5 (3.9)	6 (1.4)	

For calculating percentage, denominator was the number of subjects in each group. (127 in RV positive group and 425 in RV negative group).
[a] p-value calculated using Chi-square test.
[b] p-value calculated using Mann–Whitney U test.

Table 3
Healthcare costs, healthcare resource utilization, and impact of AGE on parents and family of children suffering from AGE.

	RV positive $N = 127$, n (%)	RV negative $N = 425$, n (%)	p-value
Direct cost (mean ± SD) (INR)			
Pre OPD	676 ± 766.3	580 ± 413.4	0.9124[a]
OPD visit (Day 1)	1428 ± 1324.5	865 ± 1225.1	<0.0001[a]
Day 1–Day 7 (subsequent to OPD visit)	1193 ± 1933.2	667 ± 1888.1	0.0066[a]
Day 8–Day 14	381 ± 3637.8	193 ± 2383.4	0.0040[a]
Overall	3177 ± 4789.1	1787 ± 4262.2	<0.0001[a]
Direct cost as per healthcare utilization[b] (mean ± SD) (INR)			
Hospitalized	7158 ± 7240.7	6895 ± 8321.1	0.3390[a]
OPD treatment	1478 ± 1015.4	1106 ± 2751.5	<0.0001[a]
Healthcare utilization			
OPD followed by hospitalization	38 (29.9)	50 (11.8)	<0.0001[c]
OPD	89 (70.1)	375 (88.2)	
Repeat outpatient visit	18 (14.2)	47 (11.1)	0.3393[c]
IV hydration	39 (30.7)	53 (12.5)	<0.0001[c]
Telephonic call to physician	4 (3.1)	35 (8.2)	0.0497[c]
Number of work days lost			<0.0001[c]
0.5–3	35 (27.6)	62 (14.6)	
≥3	9 (7.1)	11 (2.6)	
Monetary impact of availed leaves (INR)			0.0008[d]
<1000	28 (22.0)	48 (11.3)	
≥1000 to <5000	15 (11.8)	25 (5.9)	
≥5000	1 (0.8)	3 (0.7)	
Occurrence of GE in household[e]			
1	2 (1.6)	0	
2	1 (0.8)	1 (0.2)	

For calculating percentage, denominator was the number of subjects in each group. (127 in RV positive group and 425 in RV negative group).
[a] p-value calculated using Mann–Whitney U test.
[b] For "Direct cost as per healthcare utilization", number of subjects hospitalized were: ($N = 88$) (RV positive = 38, RV negative = 50); and number of subjects who attended OPD only were: ($N = 464$) (RV positive = 89, RV negative = 375).
[c] p-value calculated using Chi-square test.
[d] p-value calculated using Fisher's exact test.
[e] Includes the number of family members reporting GE symptoms from 2 weeks before the enrollment visit up to 2 weeks after enrollment visit, i.e., Day 14.

(p <0.0001) of RV positive subjects (29.9% [38/127]) were hospitalized compared with (11.8% [50/425]) RV negative subjects. Of the 38 RV positive subjects who were hospitalized, only one subject (2.6% [1/38]) was severe by Clark scale, and 35 subjects (92.1% [35/38]) were severe by Vesikari scale. Compared with RV negative subjects, a higher proportion of RV positive subjects were given IV hydration (12.5% [53/425] vs. 30.7% [39/127], p <0.0001).

3.8. Impact on parents and family

The data describing parental work loss attributed to the AGE of children are presented in Table 3. Parents/guardians of 23.6% (30/127) RV positive subjects lost 2 or more days of work compared with parents/guardians of 12.0% (51/425) RV negative subjects. We noted monetary impact of leave availed by parents/guardians for a higher proportion of RV positive children compared with RV negative children.

We determined the median value of stress score to be 5 for parents of RV positive as well as RV negative subjects through 14 days. Similarly, we also scored the stress suffered by parents when their child's disease was at its peak, and noted that at the peak of the disease, the stress levels of parents of RV positive subjects were higher compared to RV negative subjects (median values 9 vs. 8, p < 0.0001).

4. Discussion

Rotavirus disease burden studies in India have evaluated children who are hospitalized but these studies fail to represent the full burden of disease. We planned this study with a focus on enrollment of pediatric subjects with AGE when they attend private outpatient clinics in urban areas of the country. Results of this study confirm that RVGE is a major cause of AGE among Indian children in the outpatient setting as 23% (127/552) of all AGE cases were detected rotavirus positive. In present study there were some

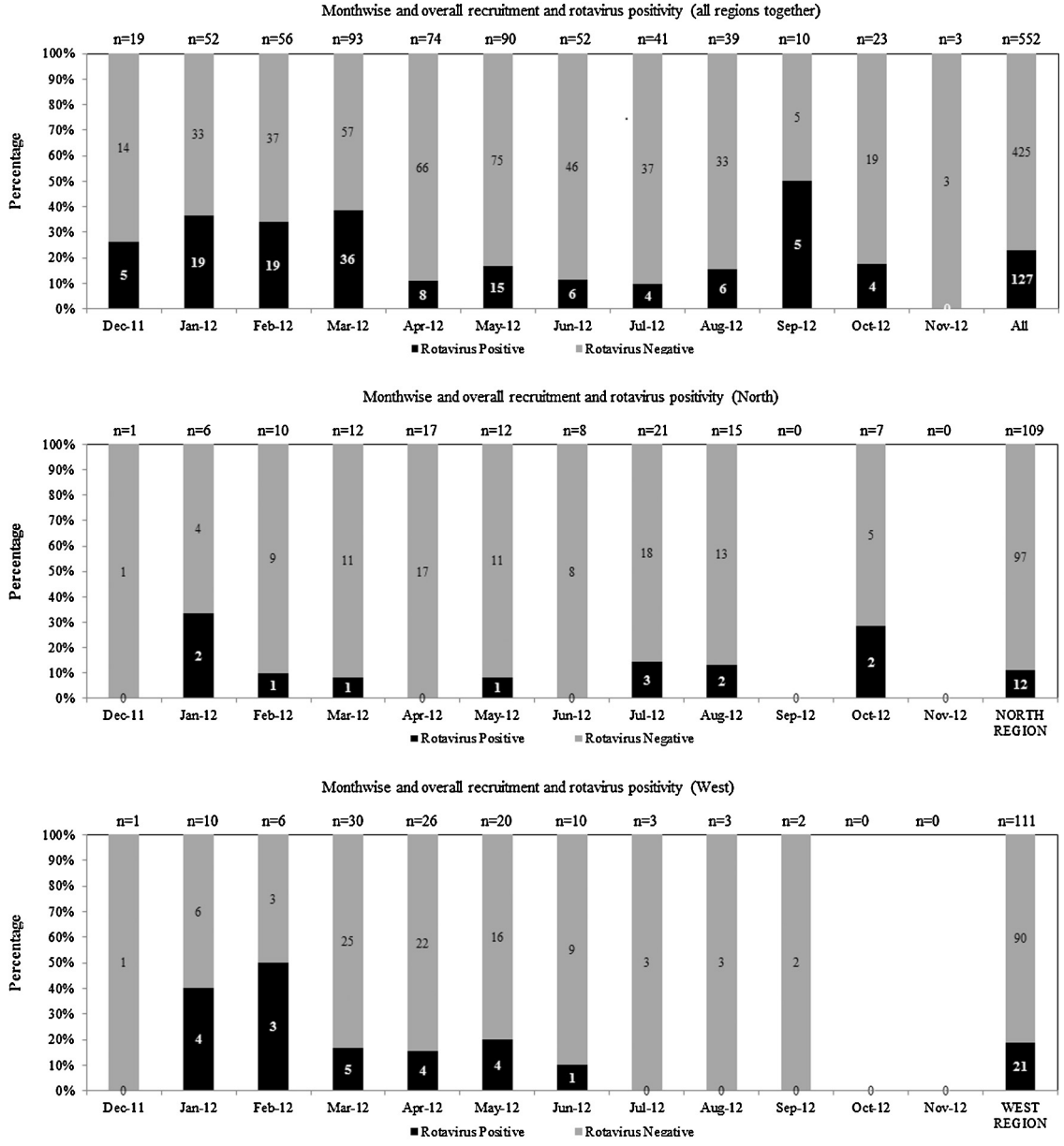

Fig. 2. Month-wise recruitment and rotavirus positivity. Footnote: the numbers at the top of bar indicate total recruitment in respective month. Bars on extreme right depict total enrollment and rotavirus detection results for respective regions.

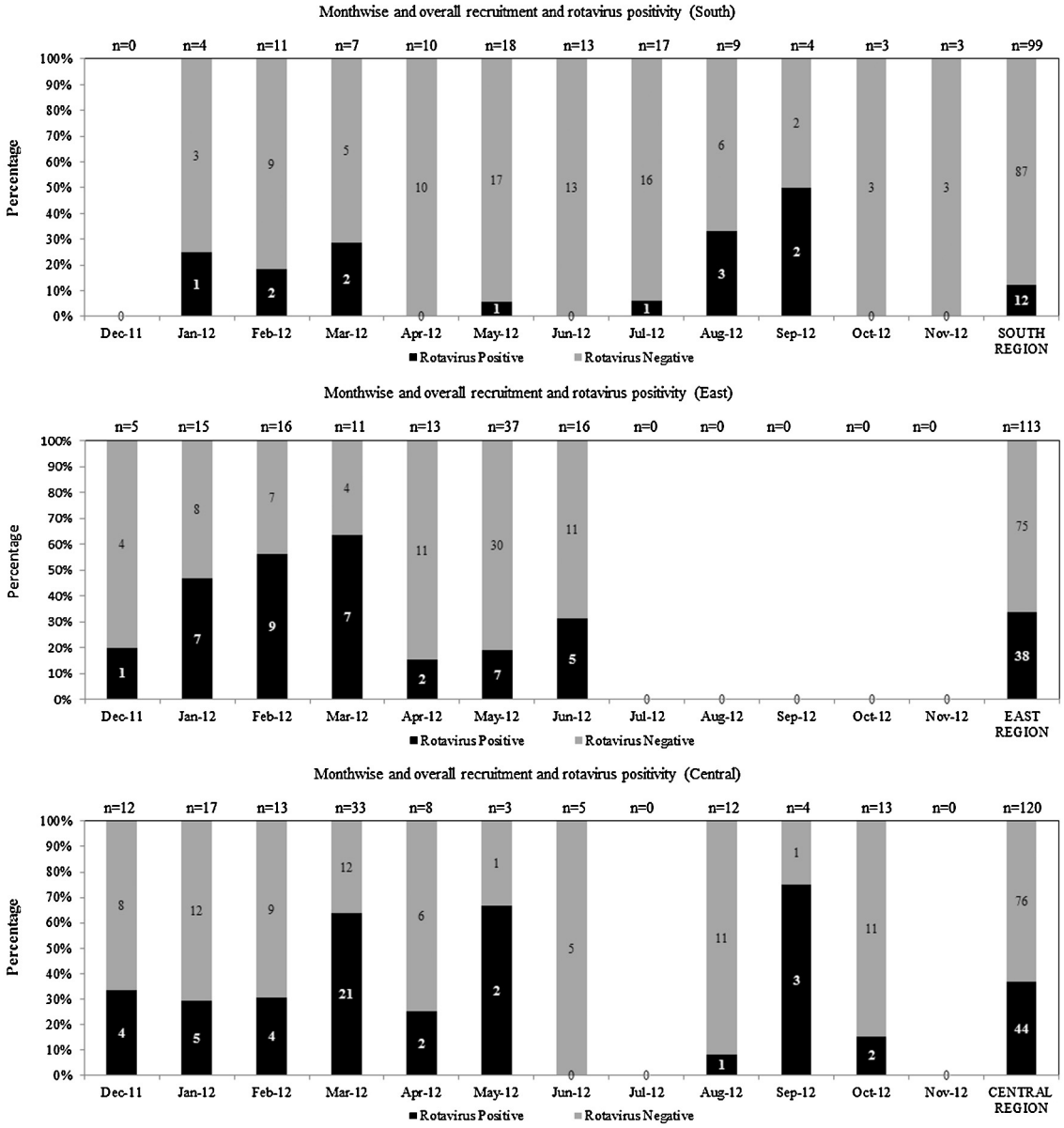

Fig. 2. (*Continued*)

cases that got hospitalized after enrollment at OPD in both rotavirus and non-rotavirus groups which were anticipated as the study was planned to enroll eligible children at OPD and treatment thereafter was as per investigator's practice. The burden of RVGE among only OPD managed AGE cases was found to be 19.2%, proportion similar to earlier two studies wherein RVGE was found in 15.5% and 22% of AGE cases treated in OPDs [15,16]. Proportion of RVGE among AGE hospitalized cases was 43.2% (38 out of 88 cases). This burden is also similar to earlier studies on rotavirus burden in hospitalized AGE cases [5,6].

We found G1 and G2 as the most common G types, P[4] and P[8] as the most common P types and G1P[8] and G2P[4] as common GP types. Some rotavirus samples could not be typed for G and/or P type. The most common G/P/GP types found in this study are similar to other Indian studies (including IRSN) conducted in children hospitalized with RVGE [2–6]. Our results show that G12 comprised 6.4% of rotavirus strains: a finding in concordance with IRSN [4,6]. G12 strain was first detected in India in 2001 and over the decade has been increasingly reported in recent Indian studies [4,6,17,18].

More than 75% of the children enrolled in the study were in the age group of less than 2 years. This reflects the age profile of diarrhea burden in India, where majority of the diarrhea episodes in children under 5 years of age are reported to occur in children of age less than 3 years [19,20]. In our study, mean age of RV positive subjects was lower compared to RV negative subjects and majority of RVGE (85%) cases occurred in children ≤24 months of age. The difference between rotavirus and non-rotavirus groups was significant w.r.t. age distribution – result similar to previous observations of the epidemiologic profile of rotavirus infection in India [4,5]. In IRSN, it was observed that the mean age of RV positive children was significantly lower than RV negative children.

In addition to younger age of RVGE subjects, our results also indicate that RV positive subjects experience severe and multiple AGE symptoms. We found that more than half of the RVGE cases were severe by Vesikari scale (77.2%) while a few were severe by Clark scale (3.9%). Similar distribution was seen in non-RVGE cases. Higher proportion of severe cases in our study may be due to late referral of the subjects to OPDs after disease onset. A 10 district survey in India by UNICEF titled "Management Practices of

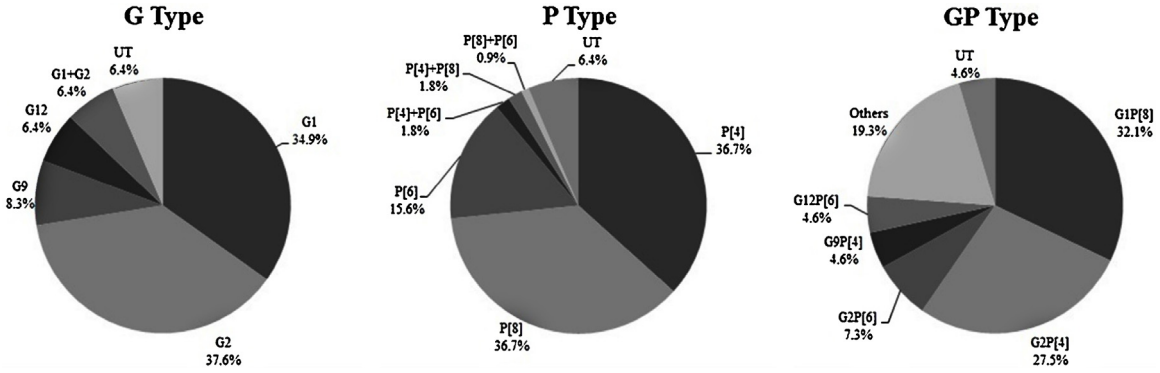

Fig. 3. Distribution of G, P, and GP types. Footnote – others include 1.8% each of the following types: G1 P[4], G9 P[8], G1 + G2 P[6], G1 + G2 P[4]; and 0.9% each of following types: G12 P[8], G1 P[6], G2 P[8], G12 P[4], G1 + G2 P[8], G2 P[4] + P[6], G2 P[4] + P[8], G9 P[8] + P[6], G1 + G2 P[4] + P[6], G9 UT, G1 + G2 UT, UT P[6], UT P[4] + P[8].

Childhood Diarrhea in India" has reported that in India in rural as well as urban areas, there is delay of at least 1 day between onset of diarrhea and time of seeking medical care outside home. The report also mentions that parents took the child outside home for managing diarrhea when child had too many stools, appeared very weak, did not eat anything, and diarrhea continued for too long [20]. It is likely therefore that majority of parents take their child to health care setting when diarrhea becomes severe. We used Clark and Vesikari scale for categorizing acute gastroenteritis into different severity levels. This categorization is dependent on multiple factors like study methodology such as where, how and when data is collected, active or passive method surveillance and frequency, timing, method of assessment in active studies. The sources of information on duration and treatment also influence the data from which a score is calculated [21]. We followed up the child till 14 days after enrollment and there was daily record of symptoms by the parents. Probably this makes the study highly sensitive and obtained the detailed information of the duration and frequency of symptoms of AGE. Finding of more severe cases by Vesikari scale as compared to Clark scale is similar to earlier studies that have used both scales. The Vesikari scale more frequently scores gastroenteritis episodes as severe as compared to the Clark scale [8,9,21].

All severe cases were not hospitalized in our study. The decision to hospitalize a child is based mainly on requirement of supervised rehydration as determined by the treating physician. In addition, factors like economic condition of parents and distance between home and healthcare setting influence decision of hospitalization [21]. It is evident from our study that in diarrheal disease and especially in RVGE, taking early treatment from health care setting would be of utmost importance to prevent complications of disease.

Our study suggests that RVGE places a considerable financial and emotional burden on parents of the affected children and they lost up to 7 days of work. The RVGE cases had higher healthcare cost and difference between RVGE and non RVGE cases was significant in OPD managed cases. Our results show that pediatric RVGE caused considerable stress for parents. This is consistent with results of a study conducted across European countries where stress scores of >5 on 10-point scale were reported irrespective of settings under which the child was treated [22].

Though study provides substantial data on RVGE in specified setting and overall proportion of RVGE is in concurrence with earlier studies, the results of this study need to be interpreted with caution because of certain important limitations. Study was conducted only in private outpatient clinics in urban areas of India and is not representative of rural and non-private healthcare settings such as government healthcare facilities or non-profit hospitals/clinics. These settings might have different rotavirus disease profile and economic impact on subjects who utilize these services may be different. It is noteworthy however that in our study, the private and urban setting has shown RVGE as important health problem, reaffirming the universal occurrence of RVGE.

IRSN data has shown that though rotavirus disease occurs throughout the year, higher proportion is observed in winter season (December–February) particularly in northern India. It has also been shown that proportion of rotavirus disease is higher in younger age and more severe cases [4]. Even in our study, when total PP population was considered, we did find that RVGE is associated with younger age, multiple symptoms, more severity of the disease as per Clark and Vesikari scale and higher proportion in the months of January–March. The enrollment period across the study sites also varied as some sites completed the study in a few months, while some sites continued the study for 11 months as the monthly enrollment was less. The enrollment criteria in our study were not restrictive as mentioned in study population above. Accordingly the difference in enrollment w.r.t. age, severity of AGE and month of enrollment across sites/regions might have led to wide variation in proportion of RVGE across regions. The overall study duration was less than 1 year; therefore annual patterns in the rotavirus strains could not be ascertained.

Despite these limits the study has obvious strengths: we used uniform protocol across the sites and well-established central laboratory support for RV diagnosis and typing; we used diary cards and questionnaires to understand the entire spectrum of the disease from its onset and also economic and psychological impact associated with it. We focused the study on RGVE disease in urban private clinics which has previously been under researched. To our knowledge this is the first well designed multicentric study to provide data on RVGE burden in urban private OPD setting among children with AGE in India.

5. Conclusion

We conclude that a high proportion of rotavirus among AGE cases attend pediatric outpatient clinics in urban areas of India. This is associated with substantial economic and psychological burden caused by RVGE. The results support that there is definite need of well tolerated and effective rotavirus vaccine for all eligible children in India.

Author's contributions

All of the authors made contributions to the conception and design of this study analyses, acquisition of data or analysis and interpretation of data. They actively participated in drafting the article or revised it for important intellectual content. The report

was critically reviewed and subsequently approved by each co-author.

Conflict of interest

Gajanan S. Namjoshi and Sudhanshu Pandey are employees of MSD. Sudhir Babji is employee of Christian Medical College, Vellore and has no conflicts to declare. Dr. S.K. Lalwani, Dr. Apurba Ghosh, Dr. Monjori Mitra, Dr. Anupam Sachdeva, Dr. Sundaram Balasubramanian, Dr. Suhas Kulkarni, Dr. V.K. Goyal were investigators in study and declare that they received investigator's grant from MSD. The investigators also declare that they have received honoraria and support from MSD and different pharmaceutical companies for their engagement in sponsored promotional and educational activities by the companies.

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