RESEARCH PAPER

Rotavirus Infections in Children Vaccinated Against Rotavirus in Pune, Western India

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Objective: To characterize rotavirus infections detected in rotavirus vaccinated children hospitalized for acute gastroenteritis.

Design: Observational, hospital-based study.

Setting: Three hospitals in Pune, Western India.

Participants: Children aged <5 years hospitalized for acute gastroenteritis during 2013-14.

Methods: Rotavirus capture ELISA was performed on all stool samples that were collected from patients following informed consent from parents. VP7 and VP4 genes of rotavirus strains were genotyped by multiplex RT-PCR. Stool samples from vaccinated children were tested for other enteric viruses.

Results: Among the 529 children, 53 were vaccinated with at least one dose of the rotavirus vaccine. There was no difference in the mean (SD) (months) age of vaccinated [14.8 (10.6)] and unvaccinated [14.4 (10.5)] children. Rotavirus positivity was significantly higher (47%) in unvaccinated than in vaccinated (28.3%) children (P=0.01). Mean Vesikari score and severe cases were significantly more in rotavirus positive than in negative children within unvaccinated group (P<0.001), while these did not differ within the vaccinated group. Rotavirus strain G1P[8] was identified as the most prevalent strain in both, vaccinated (60%) and unvaccinated (72.8%) groups. No association was found between mean Vesikari score and viral coinfections.

Conclusions: This study suggests decline in rotavirus positivity in rotavirus-vaccinated children hospitalized for acute gastroenteritis and high prevalence of G1P[8] and non-rotaviral co-infections in Pune, Western India.

Keywords: *Epidemiology, Diarrhea, Rotavirus vaccine*.

Rotaviruses continue to be the commonest cause of childhood acute gastroenteritis resulting in an estimated 24 million outpatients visits, 2.5 million hospitalizations and 450,000 deaths among children below 5 years of age worldwide [1]. Two rotavirus vaccines, Rotarix, a monovalent human rotavirus vaccine and RotaTeq, a pentavalent bovine-human reassortant vaccine have been licensed for use in several countries including India [2, 3]. With evidence of their high efficacy and safety in Americas, Europe, Australia and also in low income countries, World Health Organization (WHO) has recommended their inclusion in primary immunization programs globally [4,5]. Although studies to assess immunogenicity and safety of Rotarix and RotaTeq have been conducted successfully in India [6, 7], they have not yet been introduced in the national immunization program [8]. However, their use has been recommended by the Committee on Immunization in Children of the Indian Academy of Paediatrics [9]. In a representative survey, routine administration of rotavirus vaccine has been reported to be 9.7% by the sampled pediatricians of India in 2009-2010 [10].

During the hospital-based rotavirus surveillance being conducted at National Institute of Virology, Pune, Western India among children who were hospitalized with acute gastroenteritis in the years 2013-2014, we compared the demographic and clinical characteristics, disease severity and virological status of children who gave the history of rotavirus vaccination and others who did not.

METHODS

The criteria for enrolment of children with acute gastroenteritis and methodology used for clinical assessment and sample collection have been described earlier [11]. Accordingly, children aged less than 5 years of age admitted for acute gastroenteritis in three different hospitals in Pune, India, were enrolled in the study. Demographic and clinical data inclusive of age, date of onset and sample collection, duration and maximum number of episodes of diarrhea and vomiting, signs of dehydration, treatment and outcome of infection were recorded for all patients. Severity of diarrhea was scored on the basis of Vesikari scale [12]. Additionally, history of receipt of rotavirus vaccine was obtained from

children enrolled in the study. Efforts were made to obtain accurate rotavirus vaccination history by referring to their vaccination records and making phone calls to note the number of doses, dates of administration and type of the rotavirus vaccine. Stool samples were collected in sterile screw capped plastic containers and transported on ice to the laboratory of National Institute of Virology, Pune for detection of rotavirus antigen and strain characterization. Prior to the enrolment of a child in the study, informed consent was obtained from parent(s) or guardian. The study was approved by the institutional and hospital Ethics Committees.

Ten percent (w/v or v/v) suspension was prepared in 0.01M phosphate buffered saline (PBS) pH 7.2 from all stool samples. ELISA was performed on all suspensions using commercially available kit (Premier Rotaclone, Meridian Bioscience, Inc. USA) as per manufacturer's instructions to detect the presence of rotavirus antigen. The viral nucleic acids were extracted from 30% (w/v) suspensions of all ELISA positive stool samples using Trizol (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. The VP7 and VP4 genes were genotyped by multiplex reverse transcription polymerase chain reaction (RT PCR) according to the methods described earlier [13-15]. To determine the VP7 and VP4 genotypes of rotavirus strains non-typeable in multiplex PCR, first round PCR products were sequenced using ABI-PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster city, CA) and a ABI-PRISM 310 Genetic analyzer (Applied Biosystems) after purification on minicolumns (QIAquick: Qiagen, Valencia, CA).

The presence of noro virus (NoV), enteric adeno virus (AdV), human astro virus (HAst V) and entero virus (EV) was detected in the stool specimens collected only from rotavirus-vaccinated children by amplification of RdRp region A (126 bp), hexon (300 bp) ORF 1a (289 bp) and 5'NCR (404 bp) regions, respectively as described earlier [16-19].

Statistical analysis: Two proportions were compared using chi square test, two means were compared using Mann-Whitney test and disease severity was compared by using chi square test for 4X2 contingency table. $P<0.05$ were considered statistically significant.

RESULTS

Five hundred and twenty nine patients hospitalized for acute gastroenteritis (from January 2013 to December 2014) at three hospitals in Pune included 53 children (10%) who had received rotavirus vaccine and 476 (90%) who had not. Among the vaccinated children, 18.9%, 56.6%, and 24.5% were administered with 3, 2 and 1 doses, respectively of either of the monovalent (Rotarix) or pentavalent (RotaTeq) vaccines. Male to female ratio (1.65:1 *vs* 1.64:1) and their age distribution (0-6, 7-12, 13-18, 19-24, 25-59 months) were similar in both groups. The mean (SD) age in months of vaccinated [14.8 (10.6)] and unvaccinated [14.4 (10.5)] children hospitalized with acute gastroenteritis was comparable.

Rotavirus positivity was found to be significantly low (28.3%) in rotavirus vaccine recipients as compared to that of the non-recipients (47%) ($P=0.01$) and was higher in males as compared to females in both groups ($P<0.05$). The mean (SD) age (mo) in rotavirus positive children in vaccinated and unvaccinated groups was comparable [14.9 (9.2) and 13.5 (7.8)] ($P>0.05$). The same two groups of rotavirus positive children also showed similar clinical profile and presentation in terms of fever, history of vomiting and diarrhea, duration of hospital stay and Vesikari scores ($P>0.05$) (***Table* I**).

Within the group of children who had not received rotavirus vaccine, mean episodes of vomiting and diarrhea per day and mean Vesikari scores were more commonly observed among rotavirus positive than in rotavirus negative children ($P=0.001$, $P<0.001$ and $P<0.001$, respectively), and duration of hospital stay was longer in rotavirus negative children than in rotavirus positive children ($P=0.001$). Such analysis performed within the vaccinated group of children showed more occurrences of vomiting and more number of diarrheal episodes in rotavirus positive than in negative children (***Table* I**).

The multiplex PCR performed on rotavirus positive stool samples showed amplification of VP7 and VP4 genes in 93.3% and 100% of the strains, respectively from vaccinated group and in 98.8% and 98.3%, respectively from unvaccinated group. Among the vaccinated and unvaccinated groups of children, the most prevalent G and P types were G1 (73.3% and 75%) and P[8] (73.3% and 81.1%), respectively. Other genotypes G2, G9, G12, P[4], P[6] and P[11] were detected at low levels (0 % -13.3%) (***Fig.* 1*a*, 1*b***). Among the strains typed for both genes, G1P[8] strains were detected at the highest level in both (60% in vaccinated and 72.85 in unvaccinated) groups. Frequency of detection of other common or unusual strains that included G2P[4], G1P[6], G12P[8], G9P[8], G9P[4], G9P[6], and G12P[11] ranged between 0.0% and 7.8 % (***Fig.* 1*c***). Overall mixed infections of G and P genotypes (G1G9P[4], G1P[6]P[8], G1G9P[8], G1G9P[4] P[8], G1G2P[8], G2P[4]P[8], and G9P[6]P[8]) were detected in 2.7%-13.3% children in both groups. Only 0.5% of the strains from the unvaccinated group remained non-typeable for both genes.

TABLE I CHARACTERISTICS OF ROTAVIRUS VACCINATED AND UNVACCINATED CHILDREN WITH AND WITHOUT ROTAVIRUS GASTROENTERITIS

Variables	*Vaccinated group*			*Unvaccinated group*		
	Positive	*Negative*	*P value*	*Positive*	*Negative*	*P value*
Number	15	38	-	224	252	-
Gender- Male; *n* (%)	9 (60)	24 (63.2)	0.83	140 (62.5)	156 (61.9)	0.89
Age (mo), Mean (SD)	14.9 (9.5)	14.7 (11.1)	0.79	13.5 (7.8)	15.2 (12.4)	0.62
Fever (≥37.1^{0}C), Mean (SD)	38.2 (0.3)	38.1 (0.6)	0.67	38.1 (0.6)	38.3 (0.7)	0.06
Vomiting						
Present: No. (%)	14 (93.3)	20 (52.6)	0.005	205 (91.5)	179 (71)	<0.001
Duration (d), Mean (SD)	1 .79 (1.1)	1.65 (1.2)	0.6	1.97 (1.0)	2.16 (1.3)	0.30
Episodes/day, Mean (SD)	6.64 (5.0)	4.7 (2.8)	0.3	5.24 (3.6)	4.13 (2.7)	0.001
Diarrhea						
Duration (d), Mean (SD)	2.33 (1.0)	2.26 (1.2)	0.64	2.19 (1.1)	2.38 (1.1)	0.13
Episodes/day, Mean (SD)	13.13 (4.7)	8.18 (3.7)	0.001	11.14 (5.5)	9.22 (5.2)	<0.001
Hospital stay (d), Mean (SD)	3.6 (1.1)	4.4 (1.6)	0.19	4.07 (1.1)	4.63 (2.4)	0.001
Vesikari score, Mean (SD)	11.9 (2.4)	10.5 (3)	0.11	12.3 (2.0)	11.4 (2.7)	<0.001
Disease severity, No. (%)						
Mild	0	1 (2.6)		1 (0.4)	3 (1.2)	
Moderate	3 (20)	18 (44.7)	0.129	35 (15.6)	89 (35.3)	<0.001*
Severe	12 (80)	17 (44.7)		186 (83)	145 (57.5)	
Very severe	0	2 (5.3)		2 (0.9)	15 (6.0)	

Stool samples from both rotavirus positive and negative children from vaccinated group were examined for infections with other enteric viruses (NoV, AdV, HAstV and EV) known to cause acute gastroenteritis. Of the 53 children, 24 (45.2%) showed excretion of single or multiple viruses in the stool. Single infections of NoV, AdV, AstV and EV were detected in 5.6%, 11.3%, 1.8% and 13.2% respectively. Mixed infections of rotavirus and other enteric viruses (NoV/ EV /HAstV) were detected in six children (11.3%). In addition, mixed infections, one each of EV with HAstV and AdV with HAstV were also detected.

DISCUSSION

The present study reports the characteristics of rotavirus infections in rotavirus vaccinated and unvaccinated children, <5 years of age, hospitalized for acute gastroenteritis in Pune, Western India during 2013-2014. The data of this study indicated that although the rate of rotavirus vaccination among the enrolled children was only 10%, the vaccine recipients were less likely to have rotavirus-associated gastroenteritis as compared to the non-recipients. Further, similar disease severity scores and duration of hospital stay were recorded in rotavirus positive and negative children in the vaccinated group. In the unvaccinated group, significantly more severity score was found in the rotavirus positive as compared to the rotavirus negative children. In this (unvaccinated) group, longer duration of hospital stay was noted in the rotavirus negative as compared to the rotavirus positive children.

Most of the rotavirus-infected children from unvaccinated group of the present study were below 2 years of age, and the infections were clustered in post monsoon season as documented earlier [20]. It has been reported that shifts in the average age and seasonality pattern of rotavirus disease might take place in post vaccination period [21]. In the present study, no change in the pattern of rotavirus infections with respect to specific age and season (data not shown) was noted in the vaccinated group. Although this finding may be attributed to incomplete dosage and/or improper schedule followed for vaccination, the interpretation has limitation due to presence of small number of children in the vaccinated group.

Earlier reports from other countries have described rise in the circulation of strains other than the vaccine strains after the introduction of mono or pentavalent vaccines [22]. Virological analysis of rotavirus positive stools performed in this study for both vaccinated and unvaccinated groups was in agreement with the data reporting diversity in circulating rotavirus strains [23] and highlighted predominance of G1P [8] strains among

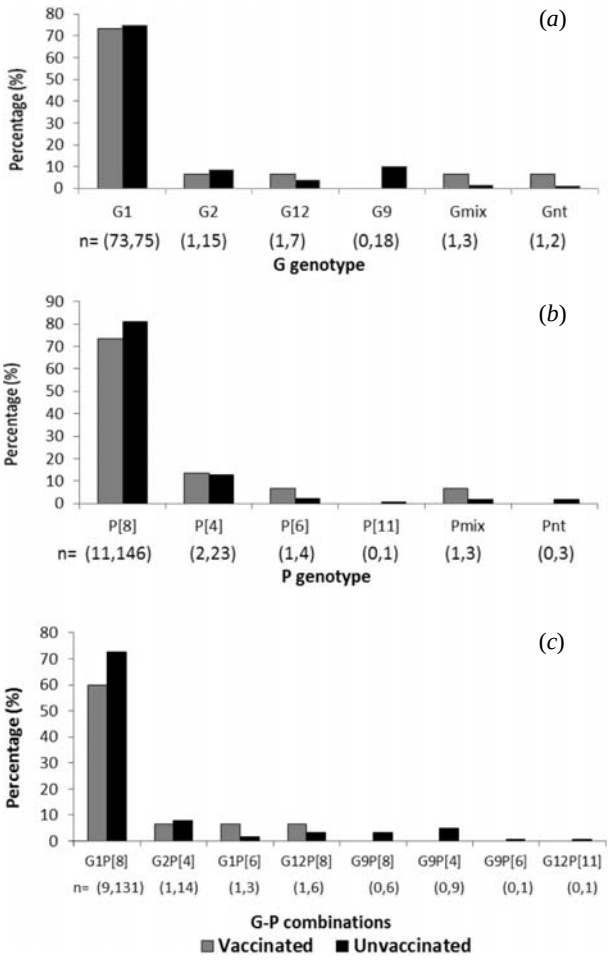

FIG. 1 *Distribution of (a) G genotypes (b) P genotypes and (c) common and unusual G-P combinations in RVA strains detected in vaccinated and unvaccinated children. The values in parentheses indicate respectively the number of children in vaccinated and unvaccinated groups positive for a particular genotype /G-P combination. Abbreviations - mix and nt stand for mixed infections and non-typeables, respectively. Gmix are G1/G2/G9 and Pmix are P[4]/P[6]/P[8].*

both groups. It may be noted that circulation of diverse rotavirus strains of a single genotype has been reported continuously in children with acute gastroenteritis from Pune and other regions of India [24,25]. On this backdrop, infections with G1P [8] strains need to be delineated by analysis of all capsid and internal genes. Further, to determine the vaccine induced pressure on genotypes, it would be necessary to have higher coverage of vaccination.

Evidence for the field effectiveness of rotavirus vaccine(s) is highly desired to enhance its eventual country-wide acceptance and use in the newer target populations. However, current unavailability of this vaccine in the national immunization program and its availability at comparatively high cost in the open market in India restrict its usage as described earlier [10]. Although, the study findings provide evidence of less likelihood of rotavirus infections in vaccinated children, it is to be noted that in this study, extent of vaccination coverage was variable and that the analysis was limited to the small number of vaccine recipients who represented city based pediatric population of Western India providing us less than the desired power for detecting a significant difference in rotavirus disease acquisition between the groups of vaccine recipients and non-recipients (78%). However, the leads do indicate a promising role of the rotavirus vaccine. In view of this, the observations made in this study need to be ascertained by examination of a large number of rotavirus vaccine recipients and non-recipients and eventually performing a classical case-control study to more objectively prove the efficacy of rotavirus vaccine in the Indian children. With rotavirus vaccine likely to be rolled out in a step-wise manner in different states of India, a systematic effort would be required to monitor the rotavirus infections and genotypes in children presenting with rotavirus vaccine and carefully document the rotavirus vaccine history to generate the data on its effectiveness in different regions of country.

Contributors: PJ: Conducted the laboratory tests, interpreted and analyzed the data and drafted the manuscript; GV: Coordinated and organized collection of clinical data and laboratory work; RG: Recorded demographic and clinical data; VK, RD and AB: Conducted clinical examination of patients admitted to the hospitals; SM: Coordinated and monitored the surveillance activity of Pune center and contributed to manuscript development; SC: Conceived and designed the study and revised the manuscript for important intellectual content. The final manuscript was approved by all authors.

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WHAT IS ALREADY KNOWN?

- Rotavirus vaccines were found to be immunogenic with good safety profile in studies conducted in India.

WHAT THIS STUDY ADDS?

- Rotavirus positivity is significantly lesser in vaccinated children admitted with diarrhea in comparison to non-vaccinated children.

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