Rotavirus Infection in the Neonatal Nurseries of a Tertiary Care Hospital in India

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Background: The majority of neonatal rotavirus infections are believed to be asymptomatic, and protection from subsequent infection and disease has been reported in neonatally infected children. In this study, we present the results of a 4-year prospective surveillance in the neonatal nurseries of a tertiary care hospital in south India.

Methods: Stool samples from neonates admitted for >48 hours either with gastrointestinal (GI) symptoms or with nonenteric pathology were screened for rotavirus. Careful assessment of clinical data was carried out. G- and P-typing for all symptomatic rotavirus positive cases and equal number of asymptomatic controls from the same month was determined by reverse transcription polymerase chain reaction.

Results: Rotavirus was detected in 43.9% of 1411 neonates, including those with and without gastrointestinal disease. Rotavirus detection was significantly higher among neonates with GI disease (55.5%) than asymptomatic neonates (44.4%) (P < 0.001). Rotavirus was seen in association with diarrhea, vomiting, feed intolerance, necrotizing enterocolitis, hematochezia, gastroesophageal reflux, and abdominal distension. Diarrhea was significantly more frequent in neonates with rotavirus infection (P < 0.001) whereas uninfected neonates developed significantly more feeding intolerance (P < 0.001). Significantly greater proportion of term neonates with GI disease were positive for rotavirus than preterm neonates (P < 0.001). G10P[11] was the most common genotype associated with both symptomatic and asymptomatic infections.

Conclusions: This study documents the high rates of rotavirus infection in the neonatal nurseries and the continuing detection of the G10P[11] strain associated with GI disease in Vellore.

Key Words: rotavirus, neonates, gastrointestinal disease, epidemiology, clinical manifestation

(Rotavirus Infection in the Neonatal Nurseries of a Tertiary Care Hospital in India)
India, and compare clinical signs between rotavirus positive term and preterm neonates.

MATERIALS AND METHODS

Study Hospital

The study was carried out exclusively in the neonatal nurseries of the Christian Medical College, a 2100-bed tertiary care hospital in Vellore, India, for a 4-year period from January 2003 to December 2006. There are approximately 250 admissions each month in the 60-bedded neonatal nurseries, of whom about 50% stay for longer than 48 hours.

Study Design. All neonates admitted in the neonatal nurseries for over 48 hours with symptoms related to the gastrointestinal (GI) tract were enrolled in the study. For every child enrolled with GI symptoms, at least one control neonate admitted for more than 48 hours with nonenteric pathology was identified and enrolled in the study. The 48-hour time period was taken as the cut off for screening as asymptomatic colonization in neonatal nurseries is known to occur with prolonged admission. Neonates with rotavirus positive stool samples were clinically further evaluated and molecular tools were used for the characterization of G and P types.

Case Definitions. Symptoms related to the GI tract were defined as one or more of the following: diarrhea, vomiting, gastroesophageal reflux, GI bleed, NEC, pneumatosis intestinalis, abdominal distension, and/or feed intolerance at any time during stay in the nursery. A neonate was defined to be symptomatic if one or more of these symptoms were seen before sample collection. A neonate was defined as asymptomatic if no GI symptoms were seen from time of admission until discharge from the nursery.

Clinical Data Collection. Careful scoring of clinical data was carried out for all neonates enrolled in the study. Information on gestational age, mode of delivery, reason for nursery admission, clinical findings, and progress were collected. Severity of disease in neonates presenting with symptoms of acute gastroenteritis was assessed using the Vesikari scoring system. Information was collected on duration of diarrhea, maximum number of stools passed per day, duration and peak frequency of vomiting, degree of fever, presence and severity of dehydration, and treatment. The disease was considered mild for scores 0–5, moderate for a score of 6–10, severe for a score of 11–15, and very severe for scores of 16–20.

Additionally, radiologic findings in all symptomatic rotavirus positive neonates in whom an abdominal radiograph was requested by the neonatologist during the course of admission were evaluated by a single pediatric radiologist blinded to the clinical findings.

Laboratory Procedures. A stool sample was collected from every neonate enrolled in the study and sent to the laboratory on the same day for testing. All samples were screened for rotavirus using an enzyme immunoassay (EIA) for detection of VP6 antigen (Rota IDEIA, Dako Ltd., UK) according to the manufacturer’s instructions. Rotavirus positive stool samples from all symptomatic neonates and an equal number of positive samples from asymptomatic neonates during the same month, if available, were taken for further characterization. Viral RNA was extracted from 10% fecal suspensions using the guanidine isothiocyanate-silica method described by Boom et al., followed by complementary DNA (cDNA) synthesis using random primers (hexamers; Pd(N)6, Pharmacia Biotech, Amersham, UK) and 400 units of Maloney murine leukemia virus reverse transcription (M-MLV RT) (Invitrogen, Life Technologies, UK). The cDNA was used as template for VP7 and VP4 (G and P) typing PCRs, using published oligonucleotide primers and methods as previously described.

Statistical Analysis. The data was analyzed using Epi Info 2002. χ², Fisher exact test tests, and Mann-Whitney U test were performed to determine the significance of differences observed between groups.

RESULTS

Rotavirus Infection in Neonatal Nurseries. A total of 1541 stool samples were collected from 1411 neonates enrolled in the study from January 2003 to December 2006. Of these, 432 samples were collected from 375 neonates with signs of gastrointestinal disease while 990 samples were collected from 924 asymptomatic neonates. One hundred and twelve neonates (119 samples) were removed from the final analysis because of lack of complete clinical information.

Rotavirus was detected in the stool samples of 619 neonates (43.9%). Detection of rotavirus was significantly higher among symptomatic neonates (208, 55.5%) than the asymptomatic group (411, 44.4%) (P < 0.001). There were no significant differences in sex, gestational age, and mode of delivery between asymptomatic and symptomatic neonates. The mean duration of hospitalization at the time of sample collection was significantly higher for symptomatic neonates than asymptomatic neonates (6.4 and 4.8 days, respectively, P < 0.001), but the 24- to 48-hour time period between development of symptoms and collection of samples contributed to this difference. Interestingly, rotavirus positive symptomatic neonates had been admitted for shorter durations at the time of sample collection (5.7 days) than rotavirus negative symptomatic neonates (7.3 days).

All children in the nurseries are less than 28 days old and the majority of neonates (85.8%) in this study were born in the same hospital, with more than 60% referred to the neonatal nurseries directly from the delivery room. An additional 11% of cases had been transferred to the nurseries within 48 hours of birth. There were no distinct temporal patterns of rotavirus positivity although peaks of rotavirus positivity were seen in January and December 2003 and >50% of samples tested positive for rotavirus during these months in 2004 and 2005.

Clinical Features. The clinical signs in rotavirus positive and negative neonates with GI symptoms are summarized in Table 1. The commonest GI symptoms seen in neonates were feed intolerance (39.5%) and diarrhea (22.4%). Diarrhea was significantly higher among rotavirus positive neonates (P < 0.001), while feed intolerance and abdominal distension were significantly higher among rotavirus negative neonates (P = 0.004 and 0.006, respectively). The overall median Vesikari score for disease severity among neonates with acute gastroenteritis indicated moderate disease that was not significantly
TABLE 1. Clinical Manifestations of Gastrointestinal Disease in Rotavirus Positive and Negative Neonates

<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>RV Positive (%)</th>
<th>RV Negative (%)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Diarrhea</td>
<td>(n = 208)</td>
<td>(n = 167)</td>
<td></td>
</tr>
<tr>
<td>Feed intolerance</td>
<td>64 (30.8)</td>
<td>20 (12.0)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Vomiting</td>
<td>25 (12.0)</td>
<td>11 (6.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>Abdominal distension</td>
<td>27 (13.0)</td>
<td>41 (24.6)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Hematochezia</td>
<td>14 (6.7)</td>
<td>15 (9.0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Gastroesophageal reflux</td>
<td>5 (2.4)</td>
<td>5 (3.0)</td>
<td>0.8</td>
</tr>
<tr>
<td>Dehydration</td>
<td>4 (1.9)</td>
<td>5 (3.0)</td>
<td>0.5</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>34 (16.3)</td>
<td>28 (16.8)</td>
<td>1</td>
</tr>
<tr>
<td>Stage I</td>
<td>28 (13.8)</td>
<td>15 (9.0)</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>3 (1.9)</td>
<td>7 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>5 (2.5)</td>
<td>6 (3.6)</td>
<td></td>
</tr>
</tbody>
</table>

All values inside parentheses indicate percentages.

Different between rotavirus positive (median severity score 6) and rotavirus negative neonates (median severity score 7, P = 0.95). Assessment of the components of the Vesikari score showed that the mean duration of diarrhea was significantly longer in rotavirus infected neonates than rotavirus negative symptomatic neonates (2.04 and 1.29 days, respectively, P = 0.04). There were no significant differences in the number of episodes of diarrhea in 24 hours, duration of vomiting and fever between both groups. However, rotavirus negative neonates had significantly more episodes of vomiting in 24 hours than rotavirus positive neonates (0.9 and 2.25 episodes/24 hours, P = 0.04). Although dehydration, diarrhea and/or vomiting were seen in 124 neonates, these symptoms were primary reasons for nursery admission only in 7 neonates. All neonates in the study had received IV fluids for treatment.

Clinical presentation of necrotizing enterocolitis was seen in 62 neonates including 34 cases in the rotavirus positive group and 26 in the rotavirus negative group. Other gastrointestinal symptoms include hematochezia, vomiting, dehydration, and gastroesophageal reflux. However, there were no significant differences between the numbers of rotavirus infected and uninfected neonates presenting with these symptoms. Thirty-eight neonates each from the rotavirus positive and negative groups had more than 1 GI symptom.

Data on status at discharge was available for 1291 neonates. There were 6 deaths in this population, none of which were caused by diarrhea. It must be noted that the number of deaths in this population may not be a reflection of the actual number of neonatal deaths as there were discharges against medical advice particularly among patients from the lower socioeconomic status.

TABLE 2. Distribution of Genotypes in Symptomatic and Asymptomatic Rotavirus Positive Cases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Symptomatic RV Pos (%) (n = 208)*</th>
<th>Asymptomatic RV Pos (%) (n = 192)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>G10P[11]</td>
<td>169 (81.3)</td>
<td>155 (80.7)</td>
</tr>
<tr>
<td>G10P[ut]</td>
<td>19 (9.1)</td>
<td>19 (9.9)</td>
</tr>
<tr>
<td>GutP[11]</td>
<td>4 (1.9)</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>GutP[4]</td>
<td>0 (0.0)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Untypable</td>
<td>16 (7.7)</td>
<td>14 (7.3)</td>
</tr>
</tbody>
</table>

All values inside parentheses indicate percentages.

*UT, untypable for VP7 or VP4.

Samples from all symptomatic neonates were genotyped during each month of the study period. An equal number of asymptomatic rotavirus positive samples, closest in date to the symptomatic samples were then chosen for comparison. During some months of the study period, there were fewer asymptomatic positive samples than symptomatic samples. Hence, whereas 208 symptomatic samples were characterized, only 192 asymptomatic controls were included for genotyping.

DISCUSSION

High rates of rotavirus infection were documented in the neonatal nurseries of a tertiary care hospital in south India with significantly higher rates of detection among neonates presenting with gastrointestinal symptoms than asymptomatic neonates. Rotavirus infections associated with gastrointestinal disease was previously seen in 63% of infected neonates from the same setting. Although the rate of symptomatic infection described in this study is lower than in the previous study, it should be noted that samples collected previously were part of a possible outbreak of rotavirus infection in the nurseries which can explain the higher number of symptomatic cases. The fact that rotavirus detection was significantly higher among symptomatic neonates during a 4-year study period is evidence for the association of rotavirus with gastrointestinal symptoms, even in nonoutbreak situations.
The data on clinical manifestations showed that diarrhea was common in neonates infected with rotavirus. The duration of diarrhea was significantly longer among rotavirus infected neonates and is similar to findings in other studies where frequent stooling was significantly higher among rotavirus infected neonates. Rotavirus infection was also seen in infants with other GI symptoms and highlights the importance of other gastrointestinal symptoms seen in neonatal rotavirus infections. It is interesting to note that vomiting was significantly lower in rotavirus infected neonates and highlights another difference between clinical presentations in neonates in comparison with older children, in whom vomiting is a classic presentation of rotavirus infection. Data on disease severity among children hospitalized with rotavirus in Vellore indicate more severe disease in older children than in neonates. However, the findings of moderate diarrheal disease in neonates in this setting are significant when considered in the context of existing beliefs that most neonatal rotavirus infections are asymptomatic.

The mean duration of hospitalization with respect to time of sample collection was significantly longer for symptomatic neonates than for asymptomatic neonates. This would probably indicate that longer duration of nursery stay may have provided a greater opportunity for development of GI symptoms. However, it must be noted that stool samples for testing of rotavirus are usually collected from symptomatic neonates about 24 hours after onset of symptoms. Hence, the duration of hospitalization for symptomatic neonates at the time of development of symptoms is likely to be similar to that of asymptomatic neonates. More importantly, the fact that rotavirus positive symptomatic neonates had been admitted for shorter durations than rotavirus negative symptomatic neonates indicates that longer duration of hospitalization may not play a role in acquiring rotavirus infection in symptomatic neonates.

An interesting finding of this study was that rotavirus infection among term neonates with GI disease was significantly higher than among preterm neonates. It is believed that infection among term neonates with GI disease was significantly lower in rotavirus positive symptomatic neonates than in asymptomatic neonates. More importantly, the fact that rotavirus positive asymptomatic neonates had been admitted for shorter durations than rotavirus negative asymptomatic neonates indicates that longer duration of hospitalization may not play a role in acquiring rotavirus infection in asymptomatic neonates.

In a study from Bangalore, where G10P[11] rotavirus has been reported in neonatal nurseries since the early 1990s, a significant reduction in rotavirus diarrhea among hospitalized children was reported after high rates of asymptomatic neonatal rotavirus infection with G10P[11] (I321 strain) in Bangalore in 1999-2000. However, our studies in a community-based birth cohort in an urban slum showed that neonates infected with G10P[11] were not protected from subsequent infection or disease. It remains to be seen whether G10P[11] infection in the neonatal nursery at Vellore, as opposed to in the community setting, will result in protection from subsequent rotaviral diarrhea.

It is interesting to consider the characteristics of a strain that can remain deeply entrenched in a specific environment, such as the neonatal nursery. Inferring from the epidemiology of this strain, it would seem that G10P[11] rotavirus is very highly transmissible, but possibly less virulent than rotaviruses of other genotypes, causing wide-spread infection but limited disease in a susceptible population. Further studies on the pathogenesis and immune response will provide key insights into factors responsible for differentially causing symptomatic and asymptomatic infections in neonates.

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