Prevalence of rotaviral diarrhoea in under-five hospitalized children in a tertiary care hospital of Eastern India

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Enteropathogens; Rotavirus; Secondary bacteremia; Under-5 children

Abstract  Background: To monitor the prevalence of rotaviral diarrhoea in under-5 children (U5C) as a retrospective study in a tertiary care hospital during 1 year.
Methods: Suspected stool samples were diagnosed for rotavirus by an enzyme immunoassay kit. The same stool samples were diagnosed for the detection of any secondary bacterial infection through routine microbiological diagnosis.
Results and conclusions: Of the total 265 stool samples, 123 were diagnosed positive with rotaviral infection, of which, 59 (50.86%) samples were from children in the age group of 0–12 months; further, 28 (41.79%), 17 (58.52%), 14 (35.71%) and 5 (46.41%) were from age groups, 13–24, 25–36, 37–48 and 49–60 months, respectively. Cases of secondary bacteremia were with Klebsiella sp., Enterobacter sp., Escherichia coli and Shigella sp. in the stool samples in age groups as given: 14 (0–12 month), 3 (13–24 month), 2 (37–48 month) and 1 (25–36 month). Of the total 123 rotaviral positive infants, 62 patients had fever and 100 patients had vomiting; while, 57, 47 and 10 patients

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had ‘mild’, ‘moderate’ and ‘severe’ dehydration, respectively. Further, 34 and 89 rotaviral positive children were with malnutrition and normal nutrition, respectively; while, 19, 89 and 15 patients were hospitalized for ≤2, 3–6, and ≥7 days, respectively. Data sets for ‘severity of dehydration’ and ‘days of hospitalization’ were statistically significant, with Kruskal–Wallis H-test, independently. Of 142 rotaviral negative patients, 27 with bacterial diarrhoea, 6 with parasitic infections, 20 with antibiotic intolerance and 31 with lactose intolerance were recorded.

Introduction

Enteropathogenic viruses, bacteria and parasites are most widespread in India, because of several reasons such as, the tropical climate itself, unclean general environment and oblivious attitude towards personal cleanliness, particularly. All these factors are active in urban slum ghettos and thickly populated dwellings consisting of both marginalized and well-healed mass. Similarly, multidrug resistant (MDR) enteropathogenic infections are one of the major causes of clinical consternation in other developing countries too. Specifically, diarrhoea is the most dominating cause of mortality in under-5 children (U5C) in most countries. Rotaviral diarrhoea in U5C is noteworthy. For example, as recorded in Kerala, India, January–May was the most prevalent period for rotaviral diarrhoea in 35.9% stool samples of hospitalized U5C. The prevalence of the infection is mostly under-reported because of the lack of laboratory confirmation in most hospitals in India. Further, according to a survey in China, rotaviral infection was reported in 151 cases per 1000 U5C. In selected African countries, a survey recorded that rotavirus was the major cause of severe diarrhoea in under-5 children, as well as infants of 3–12 months age. A study from Turkey recorded that most rotaviral gastroenteritis occurred in children aged between 24 and 36 months, and the winter season was the peak time of high prevalence. In Cameroon too, rotavirus was recorded as the major cause of child mortality.

About 0.087 million hospitalizations and 0.7 million primary health care consultations for gastroenteritis were recorded in the European Union. There were 3.6 million episodes of rotaviral infection annually, from which 23.6 million U5C suffered, with 231 death cases. In the USA too, rotavirus was noted to cause about 0.41 million medical consultations, more than 200,000 emergency visits, and about 50,000–70,000 hospital admissions per year. To sum up, 0.5 million diarrhoeal deaths from rotavirus occur per year worldwide, and in developing countries rotaviral gastroenteritis was recorded in 0.8 million cases of child mortality per year, the poor nutritional status in health care being the secondary cause of the infections.

Rotavirus is characterized by a well-known genetic diversity and the most common strains of rotavirus worldwide are serotypes, G1, G2 G3, G4 and G9. These strains are responsible for 95% paediatric diarrhoea worldwide. From the Indian serotypes diagnosed with the kit, Rotacclone using ‘enzyme linked immune sorbent assay’ with samples from 1827 enrolled children in Kerala, it was ascertained that the causative viral serotypes were, G1P, G2P and G9P. In a study from the slums of New Delhi, it was determined that, the G and P serotypes of the virus had the severity of diarrhoea in under-5 children. Around 23.5% children suffered from rotaviral diarrhoea; it was recorded that, rotavirus in stool samples was present throughout the year; around 10% diarrhoea cases were due to mixed serotypes of the virus. In a study from southern India, G1–G4 and G9 were the common rotavirus serotypes in under-5 children. With reverse transcriptase polymerase chain reaction (PCR) work, the double stranded RNA fragments from individual isolates of the virus were compared with the VP6 antigen coding gene. A Brazilian study too recorded the prevalence of G2P strains of the virus in U5C. A surveillance of the rotavirus strains in 14 countries of Sub-Saharan Africa indicated that the most common infecting serotypes were P(8) and P(6). With both urban slums and nearer marginalised tribal pockets depending on this philanthropic hospital for health care, a considerable number of diarrhoea cases are registered, which was the impetus of this surveillance. The major causative organism of diarrhoea in the U5C group of patients was found to be rotavirus in this zone.

Materials and methods

Collection and processing of samples

Stool samples of hospitalised U5C having acute watery diarrhoea were diagnosed for rotaviral infections by using the enzyme immunoassay kit, Ridascreen® rotavirus (C0901, R-biopharm AG, Germany). The same stool samples were also diagnosed for the detection of any secondary bacterial infection through routine microbiological diagnosis. Sociodemographic factors, onset and duration of symptoms, grade of dehydration and malnutrition of the patients were recorded. Categorization of dehydration as ‘no dehydration’, ‘mild dehydration’, ‘some dehydration’ and ‘severe dehydration’ for patients was done according to World Health Organization guidelines. Malnutrition status was determined according to the classification of Indian Academy of Paediatrics (IAP), based on body-weight for age; when a child-weight was more than 80% of that expected for its age; it was taken as a baby of normal nutrition.

Processing of negative samples

The rotaviral negative stool samples were further processed for determining the cause of diarrhoea. Samples were diagnosed for other microbiological organisms through culture and sensitivity test. Parasitic infections in the samples were diagnosed by routine microscopy method. Lactose intolerance was detected by Benedict test.
Results

Of the total 265 stool samples, 123 were diagnosed rotaviral positive, of which 59 (50.86%) samples were from children in the age group of 0–12 months; further, 28 (41.79%), 17 (58.52%), 14 (35.71%) and 5 (46.41%) were from age groups, 13–24, 25–36, 37–48 and 49–60 months, respectively (Table 1). Lots of 123 positive rotaviral stool samples were parallelly diagnosed for the presence of secondary bacteremia; 20 samples yielded pathogenic bacteria species, Klebsiella, Enterobacter sp., Escherichia coli and Shigella sp. The maximum number of cases with secondary bacteremia in the stool samples are given in succinct: 14 cases for 0–12 month, 3 cases (13–24 month), 2 cases (37–48 month) and 1 case (25–36 month) age groups. Stool samples from patients in the age group of 49–60 months did not have any secondary bacteremia (Table 1). The prevalence of rotaviral diarrhoea was from March to August (Fig. 1).

Clinical manifestations occurring independently in rotavirus infected patients were recorded (Tables 2 and 3). Of the 59 positive rotaviral infected patients in the age group 0–12 months, 32 patients from fever and 54 patients from vomiting suffered independently. The 95% CI values and standard error of difference being 10.137 for children with fever and vomiting clearly indicated that the prevalence of these parameters had no significant pattern. On the basis of degree of dehydration, these 59 patients were further recorded of having no dehydration, mild dehydration, some dehydration and severe dehydration for the number of patients, 2, 24, 28 and 5, respectively (Table 2). Kruskal–Wallis H test for the dehydration data set was significant at $p = 0.020$, which indicated that the degree of this symptom was crucial in rotaviral infection. Again, based on the nutritional status, of the same 59 patients, 51 patients were of under-nutrition/malnutrition and the rest 8 patients were of normal nutrition status. The 95% CI values and standard error of difference of being 8.762 for children with nutrition status clearly indicated that the prevalence of these parameters had no significant pattern. Further, basing on the days of hospitalization, of these 59 patients, 8 patients for $\leq 2$ days, 43 for 3–6 days and 8 for $\geq 7$ days were hospitalized for acute watery diarrhoea (Table 3). Kruskal–Wallis $H$ test for days of hospitalization data set was significant at $p = 0.044$, which indicated that the degree of this parameter was crucial in rotaviral infection. Likewise, the clinical features of the 28 rotaviral infected patients in the age group of 12–24 months, 14 between 25–36 months, 17 between 37–48 months and 5 between 49–60 months, were recorded (Tables 2 and 3).

Of the total 265 stool samples, 142 were negative for rotavirus. There were 57 negative samples from the patients of age group of 0–12 months. Of these 57 patients, 4 patients were diagnosed for bacterial diarrhoea, 6 were diagnosed for antibiotic intolerance, 20 were diagnosed for lactose intolerance and the rest 27 were suspected for other viral infections, which may be responsible for diarrhoea. Similarly, in the age groups, 12–24, 25–36, 37–48 and 49–60 months, stool samples negative to rotavirus were diagnosed with other causes of diarrhoea. In summary, of the 142 rotaviral negative patients, 27 patients with bacterial diarrhoea, 6 with parasitic infections, 20 with antibiotic intolerance, 31 with lactose intolerance were recorded; while the rest 8 patients were suspected for other viral infections responsible for diarrhoea (Table 4).

Discussion

It was discernible from this surveillance that infants below the age of 12 months were infected often with rotavirus in dry and summer seasons. In addition to the enzyme immunoassay kit, Ridascreen®, as here, other diagnostic methods include the use of Rotaclone and latex agglutination. These tests provide rapid sensitive/specific results, but other advanced techniques definitely include electron microscopy, culture and PCR work with RNA from pathogens.

Transmission of the rotavirus occurs via faecal to oral route by person to person contact, fomite, food, water and respiratory aerosol. The virus enters through mouth, but replicates by person to person contact, fomite, food, water and respiratory aerosol. The virus enters through mouth, but replicates in the epithelium of small intestine and has got an incubation period of less than 48 h. Rotaviral gastroenteritis results in dehydration with shock, electrolyte imbalance and increases body temperature up to 102°F, with eventual hospitalization. The course of the disease starts with an incubation period of 1–3 days. If unchecked, the infection leads to death within 3–7 days. Infants ordinarily are affected much with a wide occurrence of rotavirus in stool samples from under-5 children with diarrhoea, in 2013.
range of symptoms, fever, vomiting, dehydration, pain in abdomen, loss of weight, including frank necrotising enterocolitis. In adults however, rotaviral gastroenteritis contributes only 3–5% of clinical presentation. Admittedly, diarrhoea, dehydration and vomiting are the most common symptoms along with unexplained fever in children of age, 1–5 years. Fortunately, the clinical features of rotaviral infection are non-specific and similar to the diseases caused by other gastrointestinal pathogens; nevertheless, the latter are more severe in adults. Indeed, the first viral infection in children is severe and any subsequent infection causes less severity. Apart from severe gastroenteritis in children, rarely rotavirus is reported to cause infection of cerebrospinal fluid in patients with encephalopathy. Rotavirus infections are reported to alter phosphorylation of p70S6K, mitogen activated kinase, and myosin light chain. Induced, inflammatory agents such as, prostaglandin E2, nitric oxide or altered corticosterone levels damage villi of enterocytes in small intestine.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Distribution of rotaviral infected under-5 children basing on clinical features.</th>
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<tbody>
<tr>
<td>Age group (month)</td>
<td>Rotaviral positive patients</td>
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<tr>
<td>0–12</td>
<td>59</td>
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<td>13–24</td>
<td>28</td>
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<td>25–36</td>
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<td>37–48</td>
<td>17</td>
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<tr>
<td>49–60</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>123</td>
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</tbody>
</table>

Numbers in () are percent; numbers in [] are 95% CI values; *standard error of difference is 10.137; **the Kruskal–Wallis H test for the dehydration data set was significant at p = 0.020, degree of freedom = 3.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Distribution of rotaviral infected under-5 children basing on nutritional status and days of hospitalization.</th>
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<tbody>
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<td>Age group (month)</td>
<td>Rotaviral positive patients</td>
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<tr>
<td>Total</td>
<td>123</td>
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</tbody>
</table>

Numbers in () are percent; numbers in [] are 95% CI values; *standard error of difference is 8.7624; **the Kruskal–Wallis H test for dataset for days of hospitalization was significant at p = 0.044, degree of freedom = 2.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Distribution of rotaviral negative patients based on different clinical causes for diarrhoea.</th>
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<tr>
<td>Age group (month)</td>
<td>Rotaviral negative Patients</td>
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<td>13–24</td>
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<td>37–48</td>
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<tr>
<td>49–60</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
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Total negative samples, 142 (265–123 = 142).
the secretory component of the viral infection is linked to the activation of secretion of cholesterol ions outwards. Infection also induces the reduction of activities of alkaline phosphatase, lactase, sucrose and maltase. Thus, the thwart of intake of sodium and potassium ions and simultaneous release of chlorine ions with an imbalance of calcium ions in cells lead to the ultimate breakdown of the child. Indeed, a peptide, the NSP4 is secreted after an early infection which triggers the dominant calcium activated anionic channel. Concomitantly, the stimulation of enteric nervous systems and villus ischaemia are involved after stability of the viral RNA in epithelial cells to cause the ‘rotaviral diarrhoea’.

Mixed infections are common, and it has been demonstrated that mixed infection with entero-toxic E. coli and rotavirus caused a higher mortality rate than either diseases, individually. In a surveillance, co-infection of rotavirus and E. coli (11.1–45.5%), Salmonella sp. (0.5–4.8%), Giardia sp. (1.7–8.6%) and Shigella flexneri (3.2–8.0%) had been recorded from Iran; also children with a mixed infection had the highest incidence of severe vomiting, dehydration and fever. Mixed infections might result from unhealthy environments to a larger extent in urban slums; inocula of more diverse serotypes of rotavirus along with enteropathogens prevalent in water or contaminated food are the determinative causatives, as discussed.

Development of vaccines for the virus had an effect on the disease rate locally. Rotavirus vaccine is used with limitations in children with well healed mass as this vaccine is not as popular as the polio vaccine. With this scenario of use of vaccination, the herd mechanism may be contributing to the rate of prevalence. In India, two vaccines, Rotarix, a monovalent P1A/8/G1 vaccine (GlaxoSmithKline) and Rotateq (Merck), a pentavalent bovine human reassortant vaccine are used.

In conclusion, it was seen in this region that infant/U5C mortality could be due to rotavirus, in addition to the infection from MDR enteropathogenic bacteria, since MDR pathogenic bacteria are leitmotifs of each infectious episode in the community or hospital setting. Nevertheless, rotaviral infection originates mostly from unhygienic communities. Recorded data for severity of dehydration and days of hospitalization were supported by significance with Kruskal–Wallis H test. This geographic zone too has a widespread distribution of rotavirus, especially in dry season.

Authors’ contribution
R.S., M.D., B.K.D., R.K.L.: recruitment of patients, data analysis and processing samples; R.S., S.R.: analysis of data and writing the paper; R.S., S.R., R.N.P.: drafting of final manuscript; S.R., R.N.P.: revision of the written paper. All authors read and approved the final manuscript.

Conflict of interest
The authors declare that they have no conflict of interests.

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