Rotavirus Nosocomial Infection in Children under 5 years of age: A Preliminary study in Nepal

Sherchan JB1, Ohara H 2, Sherpa K 3, Sakurada S4, Gurung B5, Tandukar S6, Pradhan L7, Burlakoti T8, Pokharel BM9, Sherchand JB10

1Dr. Jatan B. Sherchan, MBBS. MD. Lecturer, Depatment of Medical Microbiology, Kathmandu University School of Medical Sciences, Dhulikhel, Nepal, 2Dr. Hiroshi Ohara, MD. PhD. National Center for Global Health and Medicine, Tokyo, Japan, 3Dr. Kunjang Sherpa, MBBS. Research Officer, Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal, 4Dr. Shinsaku Sakurada, MD, PhD, National Center for Global Health and Medicine, Tokyo, Japan, 5Dr. Budhi Gurung, MBBS, MD, Medical Officer, Kanti Children Hospital, Maharajgunj, Kathmandu, Nepal, 6Ms. Sarmila Tandukar MSc. Microbiologist, Tribhuvan University Institute of Medicine, Research Laboratory, 7Ms. Leela Pradhan, BSc. Nursing, Sister Incharge, Kanti Children Hospital, Maharajgunj, Kathmandu, Nepal, 8Dr. Tirtha Raj Burlakoti, MBBS, DMRD, Director, Kanti Children Hospital, Maharajgunj, Kathmandu, Nepal, 9Dr. Bharat Mani Pokharel, MSc. Ph.D. Professor and Head, Department of Microbiology, Tribhuvan University Institute of Medicine, Kathmandu, Nepal, 10Dr. Jeevan B. Sherchand PhD. Professor of Microbiology, Tribhuvan University Institute of Medicine, Chief of Public Health Research Laboratory, Kathmandu, Nepal.

Address for correspondence: Dr. J B. Sherchan, E-mail: jatansherchan@gmail.com

Abstract

Introduction: Rotavirus is one of the most common causes of acute gastroenteritis among infants and young children. The spread of rotavirus infection in pediatric wards can cause acute diarrhoea during hospitalization, and in turn, prolong hospitalization or need, rehospitalization. The aim of the study was to determine the prevalence of nosocomial infection due to rotavirus and other causal organisms among children less than 5 years of age. Methodology: A cross-sectional study between November 2009 and May 2010 was conducted among 96 pediatric patients in children hospital, Nepal. Stool samples were collected during hospitalization and up to 3 days after discharge from the hospital. Rotavirus antigens were detected by EIA and standard microbiological procedure were applied for other enteric pathogens. Results: The prevalence of nosocomial infection due to rotavirus was 30.2% (29/96). Of them, 16 (55.17%) of the 29 children with nosocomial infection during hospitalization and/or up to 72 hours after discharge developed diarrhoea. The prevalence of symptomatic cases of nosocomial infection was 55.17% (16/29), which involved 16.7% (16/96) of the study subjects, showing a prevalence of 44.82% (13/29) for asymptomatic nosocomial infection consisted of 13.54% (13/96) of the total subjects. Eleven of the 16 symptomatic subjects of nosocomial infection were affected by diarrhoea during hospitalization and five subjects after discharge. Bacteria (8.3%) and protozoa (5.2%) agents were also causing nosocomial infection in children. Conclusions: Children attending hospital may asymptptomatically carry enteric pathogens and potentially act as source of nosocomial infection. Due to the relatively high frequency of nosocomial infection in the Children Hospital of Nepal, it is necessary to follow strictly hygienic rules such as isolation of patients with diarrhoea and hand-washing before and after the examination of each patient and cleaning the examination instruments after use in each patient.

Key words: Diarrhoea, children, Rotavirus infection, Cross-sectional study, Nepal

Introduction

A global estimate suggests that 19% of total child deaths are attributable to diarrhoea and 73% of these deaths are concentrated in 15 developing countries. It is the second most common cause of death due to infectious diseases in children under five years of age and also a major cause of adult death in such
Rotavirus remains the most common cause of severe, dehydrating diarrhea among children worldwide\textsuperscript{1,2,3}. In Nepal Rotavirus burden was found in 25-50 percent of children with diarrhea among hospitalized and non-hospitalized infants and young children\textsuperscript{7,8,9}. Diarrhea is best defined as an excessive loss of fluid and electrolytes in stool (>5 g/kg of stool output per day). It is a common disease of childhood that accounts for a high percentage (10-15\%) of hospitalization in pediatric wards and annual mortality of nearly two million children worldwide\textsuperscript{10}. The major causes of acute diarrhea are infectious pathogens, with viruses being the most common. Among the viruses, rotavirus is the leading causes of diarrhea, resulting in more than 125 million episodes of acute diarrhea and 600 thousand deaths per year among children aged less than five years, worldwide.

This virus is also considered to be the most frequent etiological agent of nosocomial infections due to diarrhea\textsuperscript{11}. Twenty to 50\% of gastroenteritis caused by rotavirus is of nosocomial origin\textsuperscript{11}. This infection is more prevalent and intensive during the first two years of life\textsuperscript{12}. It is common in the colder months\textsuperscript{10}, and oral-fecal contamination is also possible. As the disease provides short-term immunity, recontamination is not uncommon. Spreading of this infection is common in hospitals and childcare centers. It is considered to be the most important factor in hospital-acquired diarrhea which increases the duration of hospitalization and/or rehospitalization. Nosocomial infections are those acquired during hospitalization and become evident after discharge\textsuperscript{13, 14}. Regarding the 48-72-hour incubation period of rotavirus, for the assessment of its role on nosocomial infection, it is necessary to examine the child up to 48 hours after discharge from hospital; this is considered as an acquired asymptomatic case.

It is important to prevent colonization of hospital staff and spread to the children admitted in the hospital. Simple preventive measures will help in prevention and control of nosocomial rotavirus infection. There is lack of information about nosocomial rotavirus infection among healthcare workers in Nepal.

The study was conducted to evaluate the prevalence of rotavirus nosocomial infections and other enteric pathogens in children less than 5 years. The study will raise awareness about nosocomial rotavirus infection among healthcare staff.

**Methodology**

A cross-sectional study was conducted during winter seasons (November 2009 and May 2010) in Kanti Children’s Hospital, Kathmandu, Nepal. The study population comprised all patients, aged 3-59 months, who had been hospitalized because of diseases other than diarrhea. Subjects were selected by single sampling from among those children who had been admitted. Patients who had negative rotavirus antigen in stool examination on the first day of admission were included in the study. If these children became rotavirus antigen-positive in stool obtained during hospitalization and or up to 72 hours after discharge, they were considered to have a nosocomial infection. Considering the prevalence of preliminary study, the necessary sample size to reach a confidence level of 95\% was calculated as 86 subjects. Clinical information included sex, age, type of disease, and length of hospitalization. Stool samples were examined for those patients who had been affected by diarrhea during hospitalization and/or up to 72 hours after discharge (except the first 24 hours) and for those children who were not affected by diarrhea during hospitalization and/or up to 72 hours after their discharge. Patients with diarrhea from 48 hours after hospitalization to 72 hours after discharge with positive antigen in their stools were considered as symptomatic cases of nosocomial infection. Stool samples were collected in a clean and sterile screw capped container by the parents and submitted along with the questionnaire and written consent. The submitted samples were kept at 4°C in the laboratory and the stool samples were processed according to the standard laboratory methods. Rotavirus infection was determined using premier Rotaclove i.e. EIA for the detection of rotavirus antigen in human fecal samples (Meridian Bioscience, Inc., USA), according to the instructions of the manufacturer.

Microscopic examination was made for the detection of oocyst, cyst and trophozoites, of protozoa and for larva or eggs of helminthes. A modified Ziehl Neelsen staining procedure was used for detecting Cryptosporidium parvum and other coccidian oocysts, including Cyclospora cayetanensis, from the stool samples. For Bacterial identification stool samples were cultured on MacConkey agar (HiMedia) for the selection of Escherichia coli isolates and use of polyvalent serotyping for identification of pathogenic E. coli. Salmonella-Shigellosa agar (HiMedia) for the selection of Shigella and Salmonella, and Thiosulfate citrate bile salt chola agar (HiMedia) for the selection of Vibrio. Alkaline peptone water was used as enrichment media for Vibrio spp. Purity plate and quality control was maintained through the experimental procedure to maintain the aseptic condition. The cultures were then incubated overnight at 37°C. All samples were tested for Vibrio spp., Shigella spp. and Salmonella spp. by using
Gram’s staining, colony morphology and biochemical properties.

Results

Out of 145 patients, 16 patients were rotavirus antigen positive during admission, 8 developed diarrhoea during 48 hours of admission and 25 patients didn’t come for follow up. Out of remaining 96 pediatric patients meeting the criteria 61 (63.54%) were boys and 35 (36.46%) were girls included in the study. The mean duration of hospitalization was 9 (Range 5-22) days.

Among 96 pediatric patients, 29 (30.2%) had positive rotavirus antigen in their stools in the first 72 hours after discharge. Of them, 16 (16.7%) were affected during hospitalization and 13 (13.5%) were affected during 72 hours after discharge.

Overall, 16 (55.2%) of the 29 children with nosocomial infection during hospitalization and/or up to 72 hours after discharge developed diarrhoea. The prevalence of symptomatic cases of nosocomial infection was 55.2% (16/29), which involved 16.7% (16/96) of the study subjects. The prevalence rate for asymptomatic nosocomial infection was 44.82% (13/29) among 13.54% (13/96) of the total subjects. Eleven of the 16 symptomatic subjects, nosocomial infection were affected by diarrhoea during hospitalization and five after discharge.

In the present study of 96 pediatric patients, other enteropathogens were identified in which 8 pathogenic bacteria and 5 protozoan parasites. These were considered as a nosocomial infection depicted in Table 1. Among other enteropathogens, bacteria (8.3%) and protozoa (5.2%) were identified ($p<0.05$).

Table 1: Distribution of nosocomial enteric-pathogens acquisition in children (n=96)

<table>
<thead>
<tr>
<th>Enteropathogens</th>
<th>No of cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus</td>
<td>29 (30.20%)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>8 (8.33%)</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (EPEC)</td>
<td>2</td>
</tr>
<tr>
<td><em>Shigella</em> species.</td>
<td>3</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella</em> species.</td>
<td>2</td>
</tr>
<tr>
<td>Protozoal Parasites</td>
<td>5 (5.2%)</td>
</tr>
<tr>
<td>Giardia lamblia</td>
<td>3</td>
</tr>
<tr>
<td><em>Cyclospora cayetanensis</em></td>
<td>1</td>
</tr>
<tr>
<td>Cryptosporidium parvum</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

The study was conducted to assess the relative frequency of nosocomial infection due to rotavirus in hospitalized children aged 3-59 months in the paediatric patients of the children hospital of Nepal. In our study, the prevalence of rotavirus infection was 30.2%, of which 16.7% were symptomatic. In Nepal, numerous studies have been performed on the prevalence of rotavirus infection in children with diarrhoea, but no study on nosocomial infection due to rotavirus associated diarrhoea in children. In our previous study, rotavirus infection was found in 30-50% with acute gastroenteritis from hospitalized and non hospitalized infants and young children$^6,9$. In urban and rural communities of Nepal the burden of rotavirus was 10-15%$^{7,8}$. Studies conducted in other countries found a prevalence of 8-33% of nosocomial infection$^{14}$. The prevalence of rotavirus-associated nosocomial infection reported in Italy was 27.7%$^{14}$, 14-16% in Australia$^{15,16}$ and 19.4% in France$^{17}$. The relative frequency of this infection is considerably higher in our study than these studies. The methods of these studies were similar to ours, and the differences in the frequencies in different studies may be due to some epidemiological parameters, such as isolation of patients with diarrhoea and following health rules in different hospitals. In our study, 16.7% of the total cases studied had symptomatic nosocomial infection due to rotavirus, which is similar with the study in Italy with a prevalence of 16.18%$^{14}$. In two other studies in France and Spain, the frequency of rotavirus-associated diarrhoea during hospitalization and before discharge was, 3.3% and 9%$^{8,17}$ respectively. In our study, 13.54% of the subjects, i.e. 44.82 % of children affected with rotavirus, were asymptomatic, whereas, in the study performed in Italy, the prevalence was 10.9%$^{14}$. This suggests that, identifying healthy control is of great importance, the real prevalence of nosocomial infection has been underestimated in many previous studies, which have only included subjects affected by diarrhoea during hospitalization.

The strength of our study is twofold, firstly, it is a cross-sectional study in which nosocomial infection episodes were carefully monitored and the data collection carried out according to a given research plan. Secondly, according to our best knowledge, it is the first study of nosocomial infection episodes among pediatric patients with rotavirus diarrhoeal diseases in Nepal. However, our study had some limitations.

The major limitation was the short duration of study (6 months). If the study could have been conducted for a whole year, it could have shown the seasonal difference. However, since rotavirus is more prevalent during the autumn and winter seasons and since the study was conducted during the colder months, the results reflect a reliable estimate of this infection. The second limitation is that the study was conducted in only one hospital, but...
considering the fact that this hospital is the main referral
hospital in Kathmandu valley of Nepal with different
subspeciality pediatric wards, the study subjects may
be considered to be a good representative sample for
the study. However, a similar study is needed in other
hospitals to highlight the extent of this problem in Nepal.

The current study showed a high frequency of
nosocomial infection due to rotavirus and that most
study subjects had acute diarrhoea, while others had
an asymptomatic infection which is suggested to be
due to contamination. These findings emphasize
the importance of nosocomial infections in pediatric
wards which, in turn, result in considerable costs
of hospitalization and treatment. As documented in
some previous studies, we also suggest that
hygienic rules should be followed more strictly in all the
wards admitting children. In the current study enteric
pathogen acquisition in hospitalized children was
8.33% with bacterial infection (E. coli, shigella spp.,
campylobacter spp. and salmonella spp.) and 5.2% with
protozoal infection. Parasites that have been implicated
in nosocomial infections include Giardia lamblia,
Cryptosporidium parvum and Cyclospora cayetanensis.
Although most nosocomial infections are bacterial,
fungal, or viral in origin, a few can be caused by parasites
mainly from stool of infected patients and particularly
from severely immunocompromised patients (those with
AIDS, etc), but such protozoan infection in the current
study remain to be confirmed and require further study.
Regarding the high potential of nosocomial infections
due to rotavirus and other pathogens, it is suggested
that children with acute diarrhoea be isolated and that
emphasis must be given toward preventive measures.

Conclusions

Children attending hospital may asymptptomatically
carry enteric pathogens and potentially act as source
of nosocomial infection. Due to the high frequency of
nosocomial infection in the Children’s Hospital in Nepal,
it is necessary to follow strict prevention and control
measures such as, isolation of patients with diarrhoea,
proper hand-washing before and after the examination
of every patient and cleaning the medical equipments
after use. This can be of great help in decreasing the
prevalence of nosocomial rotavirus infection. Moreover,
rotavirus is highly contagious so, if Government of Nepal
with the support of WHO provide rotavirus vaccine for
Nepalese children under 5 years of age, the incidence
of nosocomial rotavirus infection would reduce thereby
reducing the number of hospital cross-infections and
associated costs.

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References

pediatrics. Philadelphia: Saunders, 2004: 1081-
1093.
2. Mihalache D, Fintinaru R, Iacob M, Simonca
C. Clinical study of acute diarrhoea caused
3. World Health Organization. The world health
report 2002: reducing risks, promoting healthy life.
4. Roman Riechmann E, Wilhelm de Cal I, Cilleruelo
Pascual ML, Calvo Rey C, Garcia Garcia ML,
Sanchez-Fauquier A. Nosocomial gastroenteritis
and asymptomatic rotavirus and astrovirus
infection in hospitalized children. An Pediatr (Barc)
2004;60:337-43.
5. Lopman BA, Reacher MH, Vipond IB, Hill D, Perry
C, Halladay T et al. Epidemiology and cost of
nosocomial gastroenteritis, Avon, England, 2002-
6. Shaoxiong J, Paul EK, Robert CH, Matthew JC,
Eugene JG, Roger IG. Trends in hospitalizations
for diarrhoea in United States children from 1979
through 1992: estimates of the morbidity associated
7. Sherchand JB, Larsson S, Shrestha MP. On the
incidence of rotavirus and enteric adenovirus
diarrhoea in Kanti-children hospital and General
practitioners in Kathmandu. J Nep Med Assoc
8. Sherchand JB, Haruki K. Rotavirus diarrhoea in
children and animals of urban and rural Nepal. J
T, Yokoo M, Pandey BD, Cuevas LE, Hart A and


