Norovirus Infection in Community Children with Acute Gastroenteritis in Savar Area, Dhaka, Bangladesh

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ABSTRACT

Aims: We sought to investigate norovirus burden in patients with complications of acute gastroenteritis in community level in Bangladesh. Thus, the aim of this study was to detect the incidence of norovirus in stool samples collected from study subjects with acute gastroenteritis who attended voluntarily in different community clinics at Savar area, Dhaka, Bangladesh.

Methodology: The study enrolled patients from different community clinics in Savar area during July 2012 to December 2012. Stool specimens were collected in supplied stool container from patients as part of their diagnostic procedure. Viral RNA was extracted from the samples using the QIAamp® viral RNA mini kit (Qiagen, Germany). Real-time RT-PCR assay was conducted to identify different norovirus genogroups in the stool samples.

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Results: We detected norovirus exclusively in 23.8% (10/42) of the stool samples where rotavirus was absent. Over 80% patients were aged less than 2 years and all 10 norovirus-positive samples were detected within this age range (P = 0.17). Detection rates for norovirus was the highest in July and the lowest in November among the months covered in the study. Genogroup analysis of detected noroviruses showed 1(10%) as GI, 8 (80%) as GII and the remaining 1 (10%) as the mixture of GI and GII genogroups.

Conclusions: This study has provided baseline incidence of norovirus diarrhea in patients attended at community hospitals in Savar area, Dhaka, Bangladesh. The infections were exclusively in children aged less than two years. Norovirus genogroup-II was predominant in the community infections covered under this study.

Keywords: Norovirus; gastroenteritis; genogroups.

1. INTRODUCTION

Acute gastroenteritis affects up to 5 million children and adults worldwide [1,2]. Noroviruses are the etiology for one of the common acute nonbacterial gastroenteritis in all age group humans in both developed and developing countries [3-6]. The viruses are a group of non culturable, genetically diverse entities that belong to the member of the family Caliciviridae. Noroviruses have been accounted for about 12% (range 4.4-30.7%) worldwide hospitalization for children less than 5 years of age with severe gastroenteritis [7-9] and 200,000 deaths in this age-group in developing countries each year [10]. The infections are particularly more common in young children and elder people and impose serious impact in immunocompromised patients [4]. Transmission of these highly contagious viruses usually occurs by the fecal oral route, predominantly through ingestion of contaminated water or food, person- to-person contact, airborne transmission, and contact with contaminated surfaces, food, and water [11]. Various outbreaks reported in closed or semi closed settings such as schools, cruise ships, restaurants, hospitals or nursing homes [12]. Illness is characterized by a sudden onset of nausea, vomiting, abdominal pain, diarrhea, and occasionally, low-grade fever. The impact of noroviruses as the cause of acute gastroenteritis in both developed and developing countries has been underestimated [13], and it is fact for Bangladesh as well.

Noroviruses are approximately 38 nm long, icosahedral shaped, and contain a 7.5 Kb single-stranded positive sense RNA genome. The RNA genome encodes three open reading frames (ORFs), including ORF1 that codes for the non-structural proteins, RNA-dependent RNA polymerase (POL), helicase, and protease. ORF2 encodes a major capsid protein (VP1) and ORF3 encodes a minor capsid protein (VP2) [14]. Based on sequence homologies in their RNAs, noroviruses are mainly classified into six genogroups (GI-GVI) with a seventh that has recently been proposed [15]. Genogroups GI, GII, and GIV are found to infect humans; however, the GI and GII are the most commonly described. Only a few studies have investigated the prevalence of norovirus in hospital patients in Bangladesh [16,17], but not focused in the community sectors. This study was planned to investigate norovirus burden in patients with complications of acute gastroenteritis in community levels in Bangladesh.

2. METHODS AND MATERIALS

The majority population of Bangladesh lives in rural areas where healthcare facilities are limited. The rural peoples usually seek their medical requirements from nearby community health clinics. Savar is sub-urban area located nearly 30 km north-west of Dhaka city, where, community clinic services are popular. We enrolled patients with complications of acute gastroenteritis with nausea or vomiting in different community clinics in the area for screening norovirus infection.

Stool specimens were collected in supplied stool container from patients as part of their diagnostic procedure. Portion of each specimen was examined for the presence of norovirus. Samples that were positive with rotavirus, were excluded from the study. Verbal consents were taken from parents and/or legal guardians of the study participants for their stool samples. All the personal information of the study subjects were protected by providing unique and anonymous sample-identity codes. The sample processing and examination was carried out in the Department of Microbiology, Jahangirnagar University. The specimens were transported to the laboratory with maintaining cold chain and
phosphate-buffered saline (PBS) was used to prepare 10% (w/v) stool suspension. Viral RNA was extracted using the QIAamp® viral RNA mini kit (Qiagen, Germany) as directed by the manufacturer’s instructions and stored at -20°C. We performed real-time RT-PCR assay with norovirus type specific primers and probes to detect norovirus GI and GII targeting ORF1-ORF2 junction region of norovirus genome. The primers and probes used in this study were validated earlier [18]. In brief, a total reaction mixture (25 µl) consisting 5 µl RNA, 1 µl of GoTaq® enzyme mix 12.5 µl GoTaq® optimized buffer (Promega, USA), 0.8 and 0.2 picomol of each primer and probe was prepared. The steps of amplification cycles were: reverse transcription for 30 minutes at 55°C followed by denaturation at 95°C for 30 seconds, 45 cycles of amplification of 95°C for 15 seconds and annealing-extension at 60°C for 1 minute. The reaction was run in Thermal Cycler (Applied Biosystems 7500/7500 fast, USA), and amplification plot was analyzed by 7500 software provided with the instrument. The Fisher’s exact test was done using Graph pad prism (version 5.1) statistical software to analyse significant difference of norovirus burden before and after two-year age of the patients. P values <0.05 defined statistically significant differences.

3. RESULTS

A total of 42 patients with diarrhea were screened for carriage of norovirus using real-time RT-PCR. Overall, 23.8% (10 of the 42) stool samples yielded norovirus RNA. None of the samples were positive for rotavirus. About 62% (26/42) of the study participants were male and the remaining were female. Seven noroviruses were detected from male patients and three from females. We did not observe significant gender differences in norovirus infection in our study (p=0.71). Among the reported diarrheal patients, 83.3% (35/42) were identified in children less than 2 years old and all the 10 norovirus-positive samples were detected within this age range (P = 0.17). Only 5 samples were obtained from patients of >2 year to 5 year and 1 sample was from >5 year range. No detection of norovirus was accounted among patients of >2 years of age. Table 1 shows the rates of norovirus identification by the age of the study participants.

Norovirus were screened in diarrheal specimens in the summer months (July-October) to early winter months (November-December). Late winter and spring season were not examined under this study. Detection rates for norovirus was the highest in July and the lowest in November. Table 2 shows the monthly detection of norovirus during the study.

We had done genogrouping of norovirus in the 10 positive samples using genogroup specific primers and probes. We identified 1 (10%) GI genogroup, 8 (80%) GII genogroup and the remaining 1 (10%) was detected with the mixture of GI and GII genogroups (Fig. 1).

| Age distribution | Number of patients attended | Norovirus infected patients | % of infection |
|------------------|----------------------------|----------------------------|---------------|
| <2 year          | 35                         | 10                         | 28.6          |
| >2 year to 5 year| 5                          | 0                          | 0             |
| >5 year          | 2                          | 0                          | 0             |
| Overall          | 42                         | 10                         | 23.8          |

Table 2. Distribution of norovirus in community diarrhea patients in Savar, Bangladesh, July 2012 to December 2012

| Month        | Number of sample (percentage) | Month-wise positive sample (percentage) |
|--------------|-------------------------------|----------------------------------------|
| July         | 9 (21.4%)                     | 4 (44.4%)                              |
| August       | 12 (28.6%)                    | 2 (16.7%)                              |
| September    | 7 (16.7%)                     | 1 (14.4%)                              |
| October      | 7 (16.7%)                     | 2 (28.8%)                              |
| November     | 3 (7.1%)                      | 0 (0%)                                 |
| December     | 4 (9.5%)                      | 1 (25.0%)                              |
| Total (n)    | 42 (100%)                     | 10 (23.8%)                             |
Fig. 1. Norovirus genogroups detected from community patients from Savar area, Bangladesh

Real-time PCR (RT-PCR) assay was conducted with norovirus type specific primers and probes to identify norovirus genogroups. Accordingly, 80% of the detected norovirus belonged to GII genogroup, 10% to GI group and the remaining 10% were mixture of GI and GII.

4. DISCUSSION

Among nonbacterial pathogens, norovirus is one of the most common agents for acute gastroenteritis with significant public health impact worldwide [19,20]. Unlike rotavirus, the role of norovirus in nonbacterial gastroenteritis is seldom performed. We aimed to determine the extent whether norovirus could account for substantial proportion of diarrheal infections among patients attending community clinics in Bangladesh. This study is the first in our knowledge to document the incidence (23.8%) of norovirus among patients with diarrhea from Savar area, Dhaka. The detection rate was much more similar to the previous studies in Bangladesh [16,17,21] and also comparable to other published reports from other countries [22,23]. Among the five genogroups GI, GII and GIV have been detected in humans. We identified two of these human norovirus strains in our study patients, where GII was the most predominant genogroup. This finding is comparable to earlier published reports [20,24-28]. With the applications of molecular assays, norovirus detection rate is much higher than previously assumed. Based on age distribution, we found all positive sample in the age group less than (<) 2 years of age and similar incidence among children observed in various reports [29] using molecular techniques.

Since the late 1990s, norovirus genotype GII.4 has caused at least four global epidemics and has emerged in many parts of the world. We do not know whether our GII strains belong to GII.4, because we did not do genotyping for our norovirus strains. Further analyses of these strains are required to reveal their detail genomic profile.

Usually rotavirus infection gets more priority than norovirus or other viral gastroenteritis. As such, two commercially available rotavirus vaccines, Rotarix™ and RotaTeq™, are licensed in Bangladesh and the Government of Bangladesh has decided to include them in the routine vaccination program in coming years. It is postulated that rotavirus infection prevalence will drop after the nationwide implementation of the vaccine. Then, norovirus might take an advantage and become the most common cause of childhood diarrhea in future. Therefore, detailed studies covering epidemiology, clinical features, treatment and preventive strategies of norovirus should be taken into consideration.

Although noroviruses are well recognized as a cause of epidemic acute gastroenteritis in adults and older children in developed countries, their role as an endemic diarrheal pathogen in developing countries has still been neglected. Our study rooted the baseline data of the incidence of norovirus infection among children as an important etiologic agent of diarrhea in community level in a region of Bangladesh. The findings necessitate more studies covering other regions to build a study baseline of norovirus complications throughout the country.

Considering the impact of gastroenteritis and the real prevalence of these viruses, effective public health measures should be opted to manage and prevent this infection. Continued Surveillance on gastroenteritis patients as well as in asymptomatic controls needs to be continued for further elucidating the exact role and burden of noroviruses in diarrheal diseases.

This study has several inherent limitations. All of the participants in the study were based on specific community clinics in Savar area (Dhaka). Other patients who were treated at home or admitted in hospitals were not included in this study. Moreover, this study did not include healthy controls, therefore, the carriage of norovirus could not be ascertained in matched healthy individuals or in asymptomatic patients.
So, the results obtained from this analysis may have some variation to those treated differently or living in other regions of the country. The study covered only 6 months of a year where most of the patients were aged <2 years. Further studies may be required to assess the generalisability of our findings in different aged population groups throughout the year. The small sample size was a major constraint to convey meaningful outcomes by statistical analyses. Therefore, a prospective design of longitudinal study with higher number of study participants may provide more detail insight of norovirus infection in different seasons.

5. CONCLUSIONS

This study has provided baseline incidence of norovirus diarrhea among patients who voluntarily attended community hospitals in Savar area, Dhaka, Bangladesh. The infections were exclusively found in children aged less than two years only. Norovirus genogroup-II was predominant in the community infections examined in this study. More in-depth community level study covering other regions of the country can attest our preliminary findings.

ETHICS STATEMENT

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of helsinki competing interests.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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