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Prevalence of noro-viral diarrhea in children less than 5 years of age in Western Maharashtra

[1]R Mahesh Reddy, [2]Yasmin Muhammed, [3]Safal Muhammed MK

[1]Associate Professor, Department of Microbiology, Command Hospital, Lucknow, Uttar Pradesh, India
[2]Assistant Professor, Department of Microbiology, Command Hospital, Lucknow, Uttar Pradesh, India
[3]Assistant Professor, Department of Pediatrics, Command Hospital, Lucknow, Uttar Pradesh, India

Corresponding Author:
Dr. Yasmin Muhammed (yasi.yas33@gmail.com)

Abstract

With the introduction of *Rotavirus* vaccination, Norovirus is emerging as important pathogen responsible for viral gastroenteritis in children. Most of the Norovirus outbreaks are under reported as molecular investigations are rarely used in routine clinical practice. This study was conducted to estimate the burden of Noroviral gastroenteritis in children less than 5 years old by using multiplex real time PCR and Norovirus GII was isolated from 17% of cases.

Keywords: Noro-viral diarrhea, children less than 5 years, Western Maharashtra, *Rotavirus* vaccination, Noroviral gastroenteritis

Introduction

Norovirus is a highly diverse group of positive single stranded RNA virus from *Caliciviridae* family. Geno-groups I II and IV are pathogenic to humans causing acute gastroenteritis characterized by vomiting, fever, abdominal pain, and watery diarrhea. Norovirus has feco-oral transmission and an incubation period of 24 to 48 hours. There are 9 genotypes in genogroup I and 22 in geno-group II. Majority of the outbreaks are linked with GII.4 genotype. Norovirus requires only a very low inoculum of less than 100 viral particles to cause gastroenteritis. Hence it can cause explosive epidemics in closed settings such as daycare [1-3]. With the introduction of *Rotavirus* vaccination, Norovirus is emerging as important pathogen responsible for viral gastroenteritis in children. According to centers for disease control and prevention (CDC), about 200 million cases are seen among children under 5 years old, leading to an estimated 50,000 child deaths every year, mostly in developing countries [4].

Due to difficulty in viral culture, mainstay of identification is molecular methods. Most of the Norovirus outbreaks are under reported as molecular investigations are rarely used in routine clinical practice. Significant advances in molecular technology have led to the development of easy and rapid diagnostic tests for Norovirus [5, 6]. Early diagnosis and appropriate intervention can prevent the occurrence of outbreaks. To the best of our knowledge, there is a

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paucity of epidemiological studies on Noroviral gastroenteritis among children in India. As the coverage of Rotavirus vaccination increases, Norovirus is likely to contribute to a much larger burden of childhood acute gastroenteritis. This study was planned to estimate the burden of Noroviral gastroenteritis in children less than 5 years old by using multiplex real time PCR. The feasibility of integrating multiplex real time PCR in the diagnostic evaluation of acute gastroenteritis was also assessed as a secondary outcome.

Materials and methods

Stool samples were collected from all children less than five years of age presenting with acute gastroenteritis to Pediatric department of Armed Forces Medical College, Maharashtra June 2016 to June 2019. Demographic and clinical data were also collected along with written informed consent from parents.

Samples were collected in sterile stool containers and were immediately sent to department of microbiology for viral identification. Samples were processed within 1 to 2 hours of Collection. Nucleic acid extraction (DNA/RNA) was done immediately and stored at -70 °C in a deep freezer. Real time multiplex PCR was carried out using Roche Light cycler® LC480ll. Fast Track Diagnostics (FTD, Luxemburg) viral gastroenteritis kit was used for qualitative detection of Norovirus GI and Norovirus GII. Target gene for Norovirus GI and Norovirus GII were ORF 1-2 junction. Detection wavelength used for viruses are mentioned below in the table.

Table 1: Detection wavelength used for viruses

PP mix	Pathogen	Dye	Detection wavelength (nm)
Noro PP	Norovirus GII	Green	520
	Norovirus GI	Red	670

Any specimen displaying an amplification (exponential) trace and falling below Ct was considered positive. For Norovirus GII and Norovirus GI Ct values used were 31.9 and 34.5 respectively.

Statistical analysis

All statistical data analysis was done using SPSS Ver 20. Chi Square test was used for general categorical variables. *P* value <0.05 were considered as statistically significant. Sample size was calculated with confidence level of 95%.

Results

A total of 384 consecutive non-replicative stool samples were subjected to molecular methods by multiplex real time PCR for isolation/identification of virus. Out of 384 stool samples, 17.70% (68/384) were tested positive for Norovirus GII and none of them were positive for Norovirus GI. Figure 1 depicted below is from the PCR machine we used.

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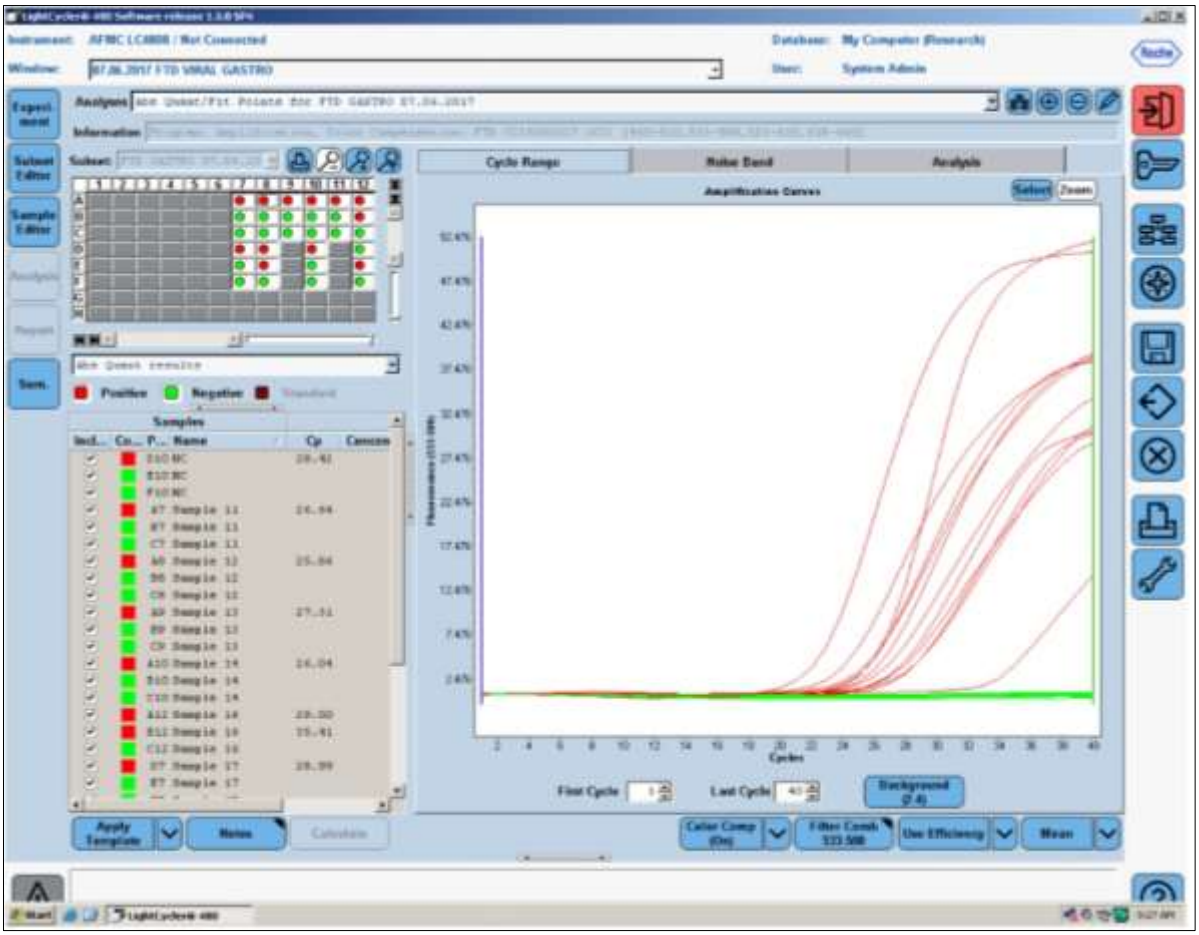

Fig 1: Clinico-demographic profile of study participants

The age of the children in the study population ranged from 1.5 months to 60 months with mean age of 18.98 months and standard deviation of 15.873. For analysis purpose, the study subjects were divided into 3 groups with open and closed intervals. All children presented with Norovirus GII diarrhea were less than 24 months of age. Using Chi-square test there was a significant association between isolation of Norovirus GII in the age group of less than 24 months (p value <0.05). The male is to female proportion was 2.4:1. Nobody required hospitalization. Socioeconomic status of study participants revealed that 64.7% (were 44/68) were from the upper lower class and 41% from lower middle class according to modified Kuppuswami's scale 2018. There was no statistically significant association between detection of viral isolates and study participant's socioeconomic status. Norovirus GII diarrhea doesn't exhibit seasonal variation, 27 cases were found during winter season and 28 cases in the summer. Only 24 participants were less than 6 months of age, out of these 16 cases with Norovirus GII diarrhea were not exclusively breast fed. No samples were visibly mixed with blood. WHO Z score system was used for assessing the nutritional status of participants. Normal nutritional status was observed in 56 participants and 4 of them had severe acute malnutrition. Fever and vomiting were observed in 76.4% (52/68) and 55.8% (38/68) of study participants respectively). We observed statistically significant association with p value <0.05 with fever and vomiting. Only 2 participants with Norovirus GII developed severe dehydration, 44/68 participants had some dehydration however this association is not statistically significant.

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Discussion

Our study has demonstrated that multiplex PCR platform can be successfully integrated into the clinical practice. It is easy to use, has a turn-around time of 4 hours and is highly sensitive and specific. Molecular diagnostic methods like Polymerase chain reaction (PCR) have promising role in diagnosis of infectious diseases like acute diarrhea. The PCR is highly sensitive and easy to perform [7].

In our study Norovirus GII was isolated from 17% of cases. Previous studies also reported comparable detection rates [8-12]. Few studies reported a lower isolation rate than our Study [13-16]. We could not isolate Norovirus GI from any of the participants. Contrary to this, studies conducted in New Delhi and Himachal Pradesh have isolated Norovirus GI [12, 13]. Children less than 2 years of age were affected in our study. Various studies conducted earlier also demonstrated same [16-19]. In our study anthropometric data of participants with and without Norovirus were similar and no significant relationship between breast feeding and Norovirus gastroenteritis were observed. Another study from South India also made same observation [18]. Children from Upper lower class (64.7%) were affected in our study. Though Norovirus is known as Winter Vomiting Disease, like other Indian studies, our study doesn't exhibit seasonal variation [17, 18]. Diarrhea, fever and vomiting were the most common presentation only two participants had severe dehydration, various studies also demonstrated the same [19, 20]. As there are no antiviral agents and vaccinations available, hand hygiene is the key tool to prevent Norovirus gastroenteritis and its outbreaks. Severe outcomes can be prevented by early assessment of dehydration and correction of fluid status. This study illustrates that developing vaccines against Norovirus will reduce the burden of acute diarrhoeal disease in early childhood.

Conclusion

Integrating multiplex real time PCR in the diagnostics of acute gastroenteritis in children is feasible and cost effective. Norovirus is an important cause of childhood acute gastroenteritis and can potentially emerge as the leading pathogen as the coverage of Rotavirus vaccination expands. Norovirus GII is the strain found in western Maharashtra as opposed to Norovirus GI found in North India. Integrating multiplex real time PCR in the diagnostics of acute gastroenteritis in children is feasible and cost effective. Norovirus is an important cause of childhood acute gastroenteritis and can potentially emerge as the leading pathogen as the coverage of Rotavirus vaccination expands. Norovirus GII is the strain found in western Maharashtra as opposed to Norovirus.

Conflicts of interest: This study does not have any conflicts of interest.

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