Journal of Clinical Virology 79 (2016) 6–9

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Norovirus detection from sera of young children with acute norovirus gastroenteritis

Leena Huhti*, Maria Hemming-Harlo, Timo Vesikari

Vaccine Research Center, School of Medicine, University of Tampere, Biokatu 10, 33520 Tampere, Finland

ARTICLE INFO

Article history:
Received 15 January 2016
Received in revised form 17 March 2016
Accepted 21 March 2016

Keywords:
Children
Gastroenteritis
Norovirus
RNAemia

ABSTRACT

Background: Antigenemia and viremia are common in rotavirus infection but only few studies have shown norovirus (NoV) RNA in the blood circulation.
Objectives: To detect NoV RNA from serum of NoV-infected children and study if NoV RNAemia correlates with clinical severity of acute gastroenteritis.
Study design: Serum specimens were collected from 176 Finnish children with acute NoV gastroenteritis. Semi-nested PCR was optimized to detect NoV capsid RNA from sera. NoV positive samples were further analyzed by sequencing.
Results: NoV RNA was found in 11/176 (6.3%) of serum specimens. NoV GII.4 was found in 8 cases and GII.3, GII.6 and GII.7 in one case each. The genotypes detected in serum were identical to findings in stools in all cases. Most of the NoV RNA detections in serum were in young children less than 18 months of age. The clinical features of NoV serum-positive cases were not different from NoV serum-negative cases.
Conclusion: NoV RNA in serum is an uncommon finding limited to young children.

1. Background

Noroviruses (NoVs) are the major viral causes of acute gastroenteritis (AGE) worldwide [1–3]. An estimated 71,000 deaths each year are associated with NoVs gastroenteritis (GE) in children worldwide [4]. In the US, NoVs are reported to cause 570–800 deaths annually, mostly among young children and elderly [5]. NoV associated GE can also be severe in specific risk groups [1,6].

NoVs are divided to genogroups that are subdivided into genotypes. The two main genogroups, GI and GII, infect humans [7]. Since 1990, GII.4 has been the predominant NoV genotype worldwide causing up to 80% of NoVGE [8]. Earlier we have shown that genotype GII.4 causes more severe gastroenteritis than other NoV genotypes in children [9].

Only few studies in children have detected NoV RNA in sera [10,11]. We found for RV that children with RV RNA in serum have more severe vomiting and fever than children with RV in stools only [12].

2. Objectives

In this study, we modified a semi-nested RT-PCR method for NoV detection from serum samples and examined the clinical features of AGE in children detected with NoV RNA in serum.

3. Study design

3.1. Clinical samples collection

The study materials were collected from children hospitalized for AGE at Tampere University Hospital from September 2006 to August 2008 [13], from September 2009 to August 2011 [3], and from September 2012 to August 2014 [14]. The ethics committee of Pirkanmaa Hospital District approved the study protocol, and parents signed an informed consent before enrolment. Stool and serum samples were stored at −20 °C until testing.

* Corresponding author.
E-mail address: leena.huhti@uta.fi (L. Huhti).

http://dx.doi.org/10.1016/j.jcv.2016.03.021
1386-6532/

We have earlier detected NoVs from stool specimens of 465 cases of AGE [3,13–16], of these 176 NoV cases had available stool and serum samples for this study.

3.2. Norovirus detection

RNA was extracted using the QIAamp® Viral RNA Minikit. cDNA synthesis was done using SuperScriptII Rnase H-Reverse Transcriptase [17]. The capsid region C of ORF2 was amplified using the primers COG2F, G2-SKR and G2-SKF [18,19]. The first PCR-reaction contained: 1 × GoTaq® Flexi buffer, 1 mM $MgCl_2$, 0.2 mM dNTPs, 0.08 mg/ml BSA, 1 μM COG2F/1 μM G2-SKR and 2.5U GoTaq® DNA Polymerase in a volume of 40 μl. 10 μl of cDNA was added to first PCR-reaction. The 40-cycle-PCR: 94 °C for 3 min, 94 °C for 30 s, 49 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. The second PCR-reaction contained: 1 × GoTaq® Flexi buffer, 1 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM G2-SKF/1 μM G2-SKR and 2U GoTaq® DNA Polymerase in a volume of 40 μl. 10 μl of the first PCR-reaction was added to second PCR-reaction. The 35-cycle-PCR: 94 °C for 3 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min. All NoV positive cases were sequenced [17].

3.3. Statistical analysis

The medians of clinical symptoms (duration of diarrhea and vomiting in days, maximum number of diarrheal stools and vomiting episodes in 24 h) were calculated from available data of NoV positive cases using Mann-Whitney *U*-test with SPSS version 23.0. Severity of NoVGE was determined using the 20-point Vesikari severity score [20].

To assess the severity of NoVGE in NoV serum-positive cases, a case-control study with ratio 1:2 was performed. For each NoV-serum positive case, two age and gender matched NoV serum-negative cases were included as controls. The clinical features of NoV serum-positive cases were compared to NoV serum-negative cases (StataCorp).

4. Results

4.1. Norovirus genotypes detected in stools

NoV genogroup GII was found in 173 cases and genogroup GI in three cases. As shown in Table 1, the main detected genotypes in stools were GII.4, GII.3, GII.6, and GII.7. The predominant NoV genotype was GII.4 (N = 135) with six different GII.4 variants followed by genotype GII.3 (N = 15) and GII.6 (N = 9).

4.2. Norovirus genotypes detected in serum

NoV RNA in serum was detected in eleven of 176 NoVGE cases (6.3%). All NoV strains detected in serum samples belonged to GII genogroup (Table 1). The most common NoV genotype in serum was GII.4, which was detected in 8 of 11 cases (73%) whereas GII.3, GII.6 and GII.7 were found in one case each. The sequences of NoV genotypes and GII.4 variants amplified from serum specimens were identical with the sequences found in stool specimens. Of the 11 NoV serum positive cases, two (GII.4 and GII.3) were coinfections with RV.

4.3. Clinical features of NoV positive cases

The age distribution of all NoVGE cases is shown in Fig. 1. The median age of all children with NoV AGE was 17 months (interquartile range (IQR), 11–39.5 months, N = 176). The NoV infected children with RNAemia were younger (median 10, IQR 7–17, N = 11) than children with NoV RNA in stools only (median 18, IQR 11–41.5, N = 165).

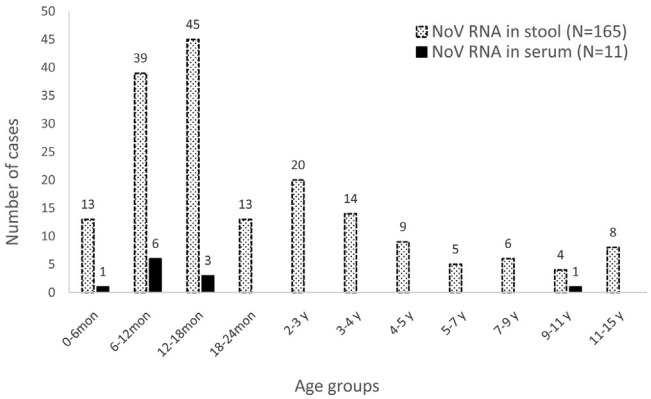

Fig. 1. Age distribution of hospitalized children with NoVGE with or without NoV RNA in serum. The numbers of NoV cases are shown above the bars.

To investigate the clinical features of NoVGE cases with or without NoV RNA in serum the two co-infection cases with RV were excluded. There were no differences between these two groups for the medians of duration of diarrhea and vomiting, maximum number of diarrheal stools and vomiting episodes in 24 h (Table 2). Children with NoV RNA detected in serum (N = 4) did not have more severe NoVGE episodes than those children who only had NoV RNA in stool (N = 55) (Table 2). To further analyze the severity of NoVGE in children with NoV RNA detected in serum, the conditional regression analysis was performed on the cases and controls. In this analysis, we did not find differences between NoV serum-positive cases compared to NoV serum-negative cases (Table 3).

5. Discussion

Rotavirus viremia among children with AGE is well documented and published [12,21]. In contrast, few studies have investigated NoV RNA in serum of NoV-infected children [10,11,22]. In this study, we found 11 NoV-infected children with NoV RNA in serum indicating RNAemia and suggesting viremia. Consistent with the observations from other studies [10,11] we also detected different NoV genotypes in serum samples confirming that NoV RNA in serum is not limited to specific NoV genotypes. The NoV strains amplified from stools and sera of the same children were identical, as also shown by others [10,11].

Some investigators have demonstrated the presence of NoV polymerase gene in serum [22]. We tried to amplify NoV RNA from serum with routinely used RT-PCR method that detects polymerase gene [17], but we did not succeed. Therefore, we modified a two-step semi-nested RT-PCR targeting NoV capsid gene. NoV RNA in serum samples has been rarely published, and we suggest that this may be due to the low sensitivity of the commonly used RT- PCR assay [17,23] for NoV RNA amplification in serum samples.

The clinical features of serum positive NoVGE cases were not more severe compared to cases with NoV found only in stools. Similar findings were reported by Fumian et al. who showed that AGE in the group that presented with NoV RNA in serum was not more severe in terms of frequency and length of diarrhea, vomiting and fever [11]. Instead, cases in children with NoV RNA present in serum have been associated with a higher viral load in stools and longer duration of hospitalization [10,11]. We only observed that NoV RNA in serum is more common in younger children, who may experience a primary NoV infection, which is likely to be associated with a higher viral load in stools. It should also be noted that this study was limited to hospitalized children, all of whom by definition have