Journal of Clinical Virology 58 (2013) 612–618

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Epidemiology and genetic diversity of human astrovirus infection among hospitalized patients with acute diarrhea in Bangladesh from 2010 to 2012

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ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form
16 September 2013
Accepted 30 September 2013

Keywords:
Human astrovirus (HAstV)
Epidemiology
HAstV-1
Genetic diversity
Diarrhea
Bangladesh

ABSTRACT

Background: Globally, human astroviruses (HAstVs) have emerged as another common cause of non-bacterial acute gastroenteritis. Limited data exist on the epidemiology and genetic diversity of HAstVs in Bangladesh.
Objective: We describe the epidemiology of HAstV-associated diarrhea among hospitalized patients, including HAstV genotypes, clinical symptoms and co-infecting pathogens.
Study design: Stool samples were collected from an ongoing diarrhea etiology surveillance during 2010–2012. HAstV was detected using RT-PCR and positive samples were subsequently tested for other common viral and bacterial pathogens. Phylogenetic analysis was performed and genotyped HAstV sequences were compared with previously reported Bangladeshi HAstV strains.
Results: Of 826 fecal specimens, HAstV was detected in 26 cases (3.1%) and the majority of these cases (92%) was observed in children under 3 years of age. For 6 out of the 26 cases (23%) no other co-infecting pathogens were observed, whereas for the 20 remaining cases (77%) a variety of other known enteric viral and bacterial pathogens were observed. Based on the overlap region between ORF1b (RdRp) and ORF2 (capsid), five different genotypes (HAstV-1, -2, -3, -5 and -6) were identified circulating during the study period, with HAstV-1 being the predominant type. Genetic analysis revealed that HAstV-1 strains detected in this study were distantly related (<90% similarity of the capsid protein on the nt level) with HAstV-1 strains previously reported from Bangladesh.
Conclusion: Our study provides an epidemiological overview and genetic diversity of HAstVs associated with acute diarrhea in Bangladesh.

1. Background

Acute viral gastroenteritis is a major public health challenge in developing countries especially in children under 5 years of age [1]. Epidemiological studies showed that human astroviruses (HAstVs) are a significant cause of infantile gastroenteritis worldwide [2,3].

The prevalence rate of HAstV infection ranged from 2% to 16% among hospitalized children with diarrhea and from 5% to 17% among community-based studies [4]. The morbidity varies depending on the season, with higher infection during winter months in temperate climates and during the rainy season in tropical regions [5].

Astroviruses (AstVs) contain a single-stranded positive-sense RNA genome divided into three open reading frames (ORFs): ORF1a

Abbreviations: HAstVs, human astroviruses; ORF, open reading frame; RdRp, RNA dependent RNA polymerase; DDSS, Diarrheal Disease Surveillance System; icddr,b, International Centre for Diarrhoeal Disease Research, Bangladesh; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcriptase polymerase chain reaction; UV, ultraviolet; nt, nucleotide; aa, amino acid.

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http://dx.doi.org/10.1016/j.jcv.2013.09.028

(non-structural protein), ORF1b (RdRp), and ORF2 (capsid protein) [6]. HAstVs are classified into at least eight genotypes (HAstV-1 to HAstV-8) [7]. The predominant genotype can vary in different geographic regions. HAstV-1 is the most common genotype identified in children, and can be responsible for up to 2.5–9% of diarrhea in hospitalized patients [8,9]. HAstV-2, -3, -4 and -5 are usually associated with diarrheal outbreaks [9–13] while HAstV-7 and -8 are relatively rare [14]. Recently, sporadic cases due to dual genotype HAstV strains, unique HAstV variants and intergenotype recombinant HAstV strains were reported from India [15,16].

In developing countries, there is an increasing evidence of severe diarrheal episodes that may be caused by bacteria–bacteria, virus–virus, parasite–parasite or virus–bacteria interactions [17]. How additional pathogens cause and contribute to the disease process is unknown, however studies demonstrated that the associations can occur between some pathogens affecting the human gastrointestinal tract [18]. For example, enteroaggregative *Escherichia coli* (EAEC) or enteropathogenic *E. coli* (EPEC) may provide a second pathogen with better conditions to invade the intestine and cause diarrhea [19]. The knowledge on polymicrobial interactions will assist to set off future research in the development of more effective diagnosis, prevention and treatment strategies.

Two papers on the prevalence of HAstV in Bangladeshi patients with gastroenteritis have been published. The first study was conducted in 1998 using ELISA, and the prevalence was found to be 8.6% [20]. The second study was conducted in 2004–2005 and HAstV was detected in 0.4% of the gastroenteritis associated cases [21]. However, the first study did not perform the genomic molecular analysis and the second study was limited to only one geographic area. Our study describes the epidemiological aspects and genetic diversity of HAstV infections associated with acute diarrhea in three different geographical regions of Bangladesh during 2010–2012.

2. Objectives

To describe the prevalence and the span of clinical symptoms associated with HAstV infections in hospitalized diarrhea patients, to describe the genetic diversity of enteric HAstVs in Bangladesh, and to investigate the prevalence of co-infecting pathogens.

3. Study design

3.1. Study sites and sample population

Samples were collected between January 2010 and December 2012 from three sites under the 'Diarrheal Disease Surveillance System (DDSS)' which, include the urban Dhaka Hospital of icddr,b (situated in the capital of Bangladesh), Matlab Hospital of icddr,b (rural site, located about 55 km from Dhaka) and the Kumudini Hospital in Mirzapur (peri-urban site, located about 60 km northwest of Dhaka) [22,23]. Detailed socio-demographic and clinical information regarding each individual was recorded in the DDSS such as onset of diarrhea, duration of diarrhea, dehydration status, vomiting, abdominal pain, fever, severity, treatment received at home and consistency of stool. Fresh whole stool specimens (at least 3 ml/g) were collected. Specimens from Dhaka and Mirzapur were transported to the icddr,b central laboratory in Dhaka within 6 h after collection with maintaining the cold chain (4–8 °C). Stool samples from Matlab were processed in the Matlab Microbiology Laboratory.

Under the DDSS, a total of 17280 individuals (n = 7840/Dhaka hospital, 4553/Matlab Hospital, 4887/Kumudini Hospital) were systematically enrolled during January 2010–December 2012. Due to funding constraints, we randomly selected 5% of the total enrolled patients per year/site for HAstV testing. Adding together, a total of n = 826 samples were tested which included 379 specimens from Dhaka, 217 from Matlab and 230 from Mirzapur Hospital.

3.2. Microbial etiologies

All the fecal samples were screened for the presence of HAstVs by reverse transcriptase PCR (RT-PCR). HAstV RNA positive samples were subsequently tested for the following viral pathogens: group A rotavirus, norovirus, enteric adenovirus (type 40 or 41), sapovirus, enterovirus, aichivirus and parechovirus using RT-PCR described elsewhere [24–29]. Bacterial pathogens: Enterotoxigenic *E. coli* (ETEC), *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp., *Aeromonas* spp., and *Campylobacter* spp., were tested in the bacteriology laboratory of icddr,b by following standard laboratory methods described elsewhere [30].

3.3. RNA extraction and RT-PCR

Viral RNA was extracted with QIAmp Viral RNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturers' instructions. RT-PCR was performed using the Qiagen one-step RT-PCR Kit (Qiagen, Hilden, Germany) using HAstV primers: forward PRECAP: 5-GGACTGCAAAGCAGCTTCGTG-3 and reverse 82b: 5-GTGAGCCACCAGCCATCCCT-3, generating a 719 bp amplicon for all classical HAstV genotypes [31]. Briefly, an initial reverse transcription step at 45 °C for 30 min, followed by 40 cycles of 94 °C for 30 s, 46 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 7 min.

3.4. Nucleotide sequencing and phylogenetic tree construction

The cycle sequencing reaction was performed using the ABI BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) and the sequencing reaction was carried out in an automated ABI 3500 xL genetic analyzer (Applied Biosystems, Foster City, USA). HAstV sequence homology was confirmed through BLAST analysis using the BLASTN program (available at: http://http://blast.ncbi.nlm.nih.gov/Blast). Sequence alignment was carried out in the BioEdit software version 7 [32]. Phylogenetic trees were constructed with the Kimura 2-parameter model and neighbor-joining methods (with 1000 bootstrap replications) using MEGA 5 [33]. Sequences were submitted to the GenBank database under the accession numbers KF420129–KF420154.

4. Results

4.1. Prevalence and clinical manifestations

During the 3-year study period, the overall rate of HAstV infection was 3.1% (n = 26/826), with 2.4% in Dhaka (n = 9/379), 3.7% in Matlab (n = 8/217) and 3.9% in Mirzapur (n = 9/230). The yearly prevalence is shown in Table 1. For 6 out of the 26 HAstVs positive cases (23%) no other co-infecting pathogens were observed whereas for the 20 remaining cases (77%) a variety of other enteric viruses or bacterial pathogens were observed. Group A rotavirus, adenovirus and norovirus were the major co-infecting viral agents while bacterial co-infections with *Shigella flexineri*, *V. cholerae* or ETEC was observed in three cases (Table 2).

4.2. Clinical manifestations

During this study, diarrhea was observed in all age groups of hospitalized cases, however, the majority (92%) of the infections were observed in children under 3 years of age (Table 3). Vomiting was observed in 4 sole (n = 4/6 [66.7%]) and 15 mixed infection