Astrovirus Infection in Young Kenyan Children with Diarrhoea

by Nicholas M. Kiulia, Jason M. Mwenda, Atunga Nyachieo, Julia K. Nyaundi, Andrew D. Steele, and Maureen B. Taylor

Institute of Primate Research, P.O. Box 24481-00502, Karen, Nairobi, Kenya

Department of Medical Virology, Faculty of Health Sciences, University of Pretoria/National Health Laboratory Service, Tshwane Academic Division (NHLS TAD), P.O. Box 2034, Pretoria 0001, South Africa

Medical Research Council (MRC), Diarrhoeal Pathogens Research Unit, MEDUNSA Campus, University of Limpopo, P.O. Box 173, Medunsa, 0204, South Africa

Abstract

Human astroviruses (HAstV) have been commonly identified worldwide as important aetiological agents of acute gastroenteritis in all age groups including the young, elderly and immunocompromised. However, limited data exist on the prevalence of this important pathogen in Kenya. The aim of this study was therefore to determine the prevalence of astrovirus (AstV) infection in Kenyan children younger than 10 years of age with diarrhoea. During the period February 1999 to September 2005, stool samples were collected from 476 children attending clinics in Nairobi (and its environs) and the Maua Methodist Hospital, Meru North, Kenya. The faecal specimens were tested by a commercial enzyme immunoassay kit for HAstV. AstV prevalence rates were found to be 6.3%. There was significantly high prevalence of AstV infection in children ≤5 years [5.3% (25/476)] than those >5 years [0.2% (1/476)] (p < 0.01). Also, we showed a significantly high prevalence of AstV infection in children of ≤5 years [5.8% (20/341)] in Nairobi (urban setting) as compared with those of similar age in Maua (a rural setting) [3.7% (5/135)] (p < 0.01). This study indicates that HAstV is an important pathogen associated with diarrhoea in young Kenyan children.

Introduction

Astroviruses (AstV), which are members of the family Astroviridae [1], are small, round, non-enveloped viruses, with characteristic five or six-pointed star-like surface structure, typically 28–30 nm in diameter [2]. The AstV viral genome is a single-stranded positive-sense polyadenylated RNA (6.8–7.6 kb in length) and contains three open reading frames (ORFs): ORF1a and ORF1b, which encode the viral protease and polymerase; and ORF2, which encodes the capsid precursor [2]. According to the reactivities of capsid proteins with polyclonal sera and monoclonal antibodies [3], human astroviruses (HAstVs) are classified currently into eight serotypes (HAstV type 1 to HAstV type 8) [4]. HAstVs were first detected in faecal samples from children with diarrhoea in 1975 [5, 6]. HAstV cause diarrhoea in infants in community setting as well as outbreaks in child care centres, hospitals and other institutions [7–10]. They have also been reported to be clinically important pathogens in the elderly [8] and immunocompromised patients [11, 12]. Since their detection, HAstVs have been implicated as one of the leading causes of infantile viral gastroenteritis worldwide [13], and in some selected geographical areas, HAstVs are second only to rotavirus as a common
cause of viral gastroenteritis in infant and children [7, 14, 15]. The occurrence of AstVs in water sources and sewage samples has also been reported [16, 17]. The common symptom of AstV infection is watery stool, which is often associated with vomiting, fever and abdominal pain [10]. Studies in rural Mexico, western Africa and southern Africa showed that AstV was a common cause of infantile gastroenteritis, suggesting that the burden of AstV disease in developing countries may be relatively high [18–21]. However, no studies documenting the prevalence of this pathogen exist in East Africa and the Horn of Africa. We, therefore, initiated a study to determine the prevalence of AstV infection in young Kenyan children. In this study, faecal specimens were collected from children ≤10 years of age with diarrhoea who presented at various clinics around Nairobi (urban area) and Maua Methodist Hospital (rural area) and the specimens were analysed for the presence of HAstV.

Materials and Methods

Sample collection
From February 1999 to September 2005 a total of 476 children with diarrhoea ≤10 years of age attending clinics around Nairobi and its suburbs, and Maua Methodist Hospital were enrolled in this study as part of a public health programme. All specimens were stored at 4°C until analysis, with an aliquot of each specimen stored at –20°C for further reference.

Astrovirus detection
A 10% suspension of fresh stool in sample diluent [phosphate buffered saline (PBS), pH 7.4] was made and tested for the presence of AstV antigen using a commercial AstV antigen detection enzyme immunoassay (Dako IDEIA™ astrovirus kit, DakoCytomation, UK). The procedures for detection were performed according to the manufacturer’s instructions. All the specimens whose optical density reading (OD) was above the cut-off value (calculated as follows: 0.100 + negative control OD unit) were considered as positive as defined by manufacturer. A positive control (a suspension of AstV positive stool in PBS) and negative control (PBS alone) were provided with the kit and were used as quality controls to assess assay performance.

Statistical analysis
Analysis of the HAstV infection rates in children according to age and setting (rural and urban) was done using Fisher’s exact test using StatView software (SAS Institute). Differences with p-values >0.05 were considered not significant at 95% confidence interval (CI).

Results
The overall prevalence of AstV infection was found to be 6.3% (30/476) of the samples analysed (Table 1). The infection rates according to age and the setting (rural and/or urban) in which the samples were collected are presented in Tables 1 and 2. In our study the majority of children infected with AstV were ≤5 years of age (Table 1). Most (50.0%) of the infected children were <3 years of age, while 26.8% (128/476) of the patients were of unknown age and

<table>
<thead>
<tr>
<th>Age/setting</th>
<th>Prevalence of HAstV in Maua (rural setting) and Nairobi (urban setting); Kenya</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5 years</td>
<td>(3.7) 5/135²</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>–</td>
</tr>
<tr>
<td>Age unknown</td>
<td>(0.0) 0/135</td>
</tr>
<tr>
<td>Total positive</td>
<td>(3.7) 5/135²</td>
</tr>
</tbody>
</table>

Similar superscript alphabets show statistical significant difference (Fishers’ exact test p < 0.05).

TABLE 2

Prevalence of HAstV in Maua (rural setting) and Nairobi (urban setting); Kenya

<table>
<thead>
<tr>
<th>Age group samples (months)</th>
<th>Number of samples tested</th>
<th>Astrovirus positive n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>2</td>
<td>1 (50.00)</td>
</tr>
<tr>
<td>3–5</td>
<td>29</td>
<td>5 (17.20)</td>
</tr>
<tr>
<td>6–8</td>
<td>32</td>
<td>2 (6.25)</td>
</tr>
<tr>
<td>9–11</td>
<td>22</td>
<td>1 (4.54)</td>
</tr>
<tr>
<td>12–17</td>
<td>64</td>
<td>5 (7.81)</td>
</tr>
<tr>
<td>18–23</td>
<td>30</td>
<td>5 (16.66)</td>
</tr>
<tr>
<td>24–35</td>
<td>63</td>
<td>3 (16.66)</td>
</tr>
<tr>
<td>36–47</td>
<td>38</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>48–60</td>
<td>59</td>
<td>3 (5.10)</td>
</tr>
<tr>
<td>Above 60</td>
<td>9</td>
<td>1 (11.11)</td>
</tr>
<tr>
<td>Age unknown</td>
<td>128</td>
<td>4 (3.12)</td>
</tr>
<tr>
<td>Total</td>
<td>476</td>
<td>30 (6.30)</td>
</tr>
</tbody>
</table>
accounted for 3.12% of the positive cases of AstV infection (Table 1). There was significantly higher prevalence of AstV infection in children of ≤5 years [5.3% (25/475)] than those of >5 years [0.2% (1/476)] (p < 0.01) (Table 2). Nine children were >5 years of age of which only one (11.1%) was positive for AstV (Table 1). A comparison of the two settings, i.e. Maua (rural setting) and Nairobi (urban setting), showed a significantly higher AstV infection rate in Nairobi [7.3% (25/341)] than in Maua [3.7% (5/135)] (p < 0.01) (Table 2). There was significantly higher prevalence of AstV infection in children ≤5 years [5.8% (20/341)] in Nairobi (urban setting) as compared to those of similar age in Maua (a rural setting) [3.7% (5/135)] (p < 0.01) (Table 2).

Discussion

Improvement of diagnostic methods has lead to detailed studies on the epidemiological and clinical aspects of AstV infection [22]. HAstV infection occurs throughout the world both in temperate climates [23], and in tropical climates [2]. AstV infection both in developed and developing countries have been associated with 4–10% of endemic diarrhoeal episodes in children [24–30]. However, in few cases a high prevalence of up to 26% of all diarrhoea episodes have been reported [18, 27]. Epidemiological studies carried out in different locations in the world have reported HAstV prevalence rates of 2–16% among hospitalized children with diarrhoea and 5–17% among children with diarrhoea in community based studies [31].

In our study, AstV antigen was detected in 6.3% specimens collected from children with diarrhoea ≤10 years of age. This prevalence is similar to other studies in both developed and developing countries [32]. A study conducted in the USA, targeting the same age group (<10 years) reported a prevalence of 6.8% of community-acquired AstV infection and 16.2% of cases occurring as a result of nosocomial infection [32]. Whereas in developing countries HAstV infection have been shown to vary in different region: 6.3% in Mexico [33] and in Africa; 7% in South Africa [20], 7% in Tunisia [34]. These results suggest that AstV infection is common in both developed and developing countries. The prevalence of AstV infection in this study was strikingly age related with the majority of children infected being ≤5 years (≤60 months old). Most (50.0%) of them were <36 months old. This finding is similar to the previously reported prevalence in 6–12 months old infants [3, 24, 35]. A comparison of HAstV infection according to setting indicates that HAstV was detected more frequently among children in urban setting than in rural setting [7.3% (25/341)] vs. [3.7% (5/135)] (p < 0.01). The reasons for this difference are unknown, but could be due to person-to-person exposure to HAstVs rather than the differences in hygienic conditions, since the latter has been reported not to have an influence infection rates as shown by the similarity in AstV prevalence both in developed (more hygienic environment) and in developing (less hygienic condition) [19, 30]. Further studies are needed to determine the genotype diversity and the seasonal distribution of HAstV infection in Kenya. To further understand the epidemiology of AstV infection and the serotypes circulating in Kenya, additional studies are needed. In conclusion, AstV appears to be an important cause of viral diarrhoea in young children in Kenya and this study has demonstrated AstV infection is of major public health importance in Kenya.

References