Molecular characterization of G1 human rotaviruses detected in children from Belém, Pará, Brazil

Caracterização molecular de rotavírus humanos do tipo G1 detectados em crianças de Belém, Pará, Brasil

Caracterización molecular de rotavirus humano tipo G1 detectado en niños de Belém (Estado de Pará, Brasil)

ABSTRACT

Rotavirus is responsible for 40% of gastroenteritis infections worldwide, resulting in 611 thousand deaths annually among infants and young children. The aim of the present study was to perform molecular characterization of strains of the most common circulating rotavirus genotype (G1), which was obtained from children participating in studies previously conducted in Belém, northern Brazil over a 21-year period (1982 to 2003). G1 type rotavirus was detected by polyacrylamide gel electrophoresis, enzyme immunoassay and by reverse transcription polymerase chain reaction for the VP7 and VP4 genes. Of 798 specimens that were found to be positive for rotavirus, 330 (41%) had G1-specificity by EIA using monoclonal antibodies. A total of 148 G1 strains were analyzed by reverse transcription polymerase chain reaction. Electropherotypes and P genotypes characterization of G1 rotavirus occurred at frequencies of 78% and 88%, respectively. Three long electropherotype varieties were identified, with the L1 variety the most frequently found (79%). The G1P[8] combination was the most frequent, responsible for 64% of cases. Mixed infections of G1P[6]+P[8], G1P[4]+P[8], G1P[4]+P[6] and G1P[4]+P[6]+P[8] were found in 11 (7%), 11 (7%), 3 (2%) and 1 (0.6%) samples, respectively. One sample displaying a mixed G1+G4 infection was found. To our knowledge, this is the first study to focus on G1 rotavirus molecular characterization in Brazil. Our findings provide information that will allow a better understanding of the molecular diversity of G1 rotavirus infections in our region.

Keywords: Gastroenteritis; Rotavirus Infections; Genetic Variation.

INTRODUCTION

Group A rotavirus (RV-A) is the most common etiological agent of severe gastroenteritis worldwide. It is responsible for 40% of gastroenteritis infections and results in 611 thousand deaths annually among infants and young children, mostly in the poorest countries. The global magnitude of rotavirus disease is widely recognized; in fact, each child will have an episode of rotavirus gastroenteritis before 5 years of age.\(^\text{24,25}\)

Rotavirus belongs to the Reoviridae family and is classified into seven groups (A-G) and four subgroups (I, II, I+II, and non I/II) according to the specificities of epitopes on the inner layer capsid VP6 protein. The rotavirus genome contains 11 segments of double-stranded RNA (dsRNA) inside the core of a triply-layered capsid. The outer capsid is composed of two structural proteins, VP4 and VP7, which define the P (protease-cleaved protein) and G (glycoprotein) genotypes, respectively. Based on the mobility of the 11 gene segments in polyacrylamide gels, rotaviruses can be identified as long, short or super-short electropherotypes.\(^\text{11}\)

To date, at least 23 G and 30 P types have been identified based on sequence analysis of the VP7 and VP4 genes, respectively; however, few genotypes are known to cause infection in humans.\(^\text{5,6,11,31,32}\) Although theoretically a high number of G/P combinations are possible, epidemiological studies worldwide have documented the circulation of five major types: G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].\(^\text{6,7,11,19,29}\)
G1 rotavirus is the most prevalent genotype, and it has been detected in frequencies ranging from 36% to 74% in different regions of the world. G1P[8] strains represent approximately 65% of rotavirus types identified globally. Castello et al. have recorded G1P[8] strains in 40% of rotavirus infections in Latin America. Recently, Leite et al. reported that G1 strains were detected in 43% of cases during the pre-vaccine period in Brazil; by contrast, after vaccine introduction this genotype was reduced to 3% of rotavirus infections due to a large predominance of G2P[4] strains.

Sequence analysis of the VP7 gene of G1 human rotavirus strains in Italy has revealed the existence of at least three VP7 genetic lineages. These antigenic variants might be responsible for the continuous circulation of G1 rotavirus. Phan et al. analyzed the VP7 gene of G1 rotavirus strains collected around the world and have suggested a novel nomenclature that includes 11 lineages and 17 sublineages.

The aim of the present study was to perform molecular characterization of G1 rotavirus strains obtained from children participating in various studies previously conducted in Belém, northern Brazil over a 21-year period (1982 to 2003).

MATERIALS AND METHODS

PATIENTS AND CLINICAL SPECIMENS

Specimens obtained from five viral gastroenteritis studies conducted in Belém, Brazil, between December 1982 and October 2003, were analyzed for rotavirus detection. Of 798 specimens that were found to be positive for rotavirus by enzyme immunoassay (EIA), 330 (41%) were G1 typed by EIA using monoclonal antibodies. A total of 148 G1 strains were analyzed by reverse transcription polymerase chain reaction (RT-PCR) to confirm these results. This analysis involved all the G1 strains detected in Studies A, B, C and D, and 20% of the cases from Study E. This study was approved by the Institutional Ethical Review Board from Evandro Chagas Institute, Health Surveillance Secretary.

RNA EXTRACTION

Rotavirus dsRNA was extracted from 10% fecal suspension by using guanidinium isothiocyanate-silica nucleic acid extraction as described previously by Boom et al.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

The RNA profile was analyzed by PAGE with silver staining as described previously.

CHARACTERIZATION OF G AND P ROTAVIRUS GENOTYPES BY REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

The VP7 genotype was determined by using reverse transcription followed by multiplex PCR as previously published. Two different sets of type-specific primers were used for G genotyping: pool A, which contained G1 (9T1-1), G2 (9T1-2), G3 (9T-3P), G4 (9T-4) and G9 (9T-B) specific primers; and pool B which contained primers specific for the G5 (FT5), G6 (DT6), G8 (HT8) and G10 (ET10) genotypes.

Determination of VP4 genotype was performed by RT-PCR followed by nested-PCR, as described by Gentsch et al. Briefly, the full-length VP4 gene was reverse transcribed and a fragment of 876 base pair (bp) was amplified. The primers utilized were specific to P[8] (1T-1), P[4] (2T-1), P[6] (3T-1) and P[9] (4T-1) genotypes.

RESULTS

MOLECULAR EPIDEMIOLOGY OF G1 ROTAVIRUS INFECTION

The highest frequency of G1 rotavirus was found in 2003 during a pediatric hospital-based survey (2003) (68%, 232 of 343), and the lowest was seen during a survey involving hospitalized children (1998 to 2000) (11%, 31 of 281) (Table 1). The median age of the 148 G1-infected children was 13 months (range, 2 month–3 years). Of these, 70 (47%) were <1 year of age, 68 (46%) were 1–2 years of age, and 10 (7%) were >2 years of age.

<table>
<thead>
<tr>
<th>Study, short title</th>
<th>Characteristics</th>
<th>Period</th>
<th>Age group</th>
<th>RV positive/samples collected</th>
<th>G1 frequency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Longitudinal Study</td>
<td>Community-based</td>
<td>December 1982 to March 1986</td>
<td>0 – 3 years</td>
<td>36/441 (8%)</td>
<td>25% (9/36)</td>
<td>Linhares (1989)</td>
</tr>
<tr>
<td>B, RRV-TV trial</td>
<td>Double-blind placebo-controlled trial</td>
<td>June 1990 to June 1992</td>
<td>1 month – 2 years</td>
<td>90/1990 (4%)</td>
<td>49% (44/90)</td>
<td>Linhares (1996)</td>
</tr>
<tr>
<td>C, Nosocomial/community infection</td>
<td>Hospital-based</td>
<td>November 1992 to May 1994</td>
<td>0 – 5 years</td>
<td>48/290 (16%)</td>
<td>29% (14/48)</td>
<td>Gusmão (1999)</td>
</tr>
<tr>
<td>D, Hospital-based surveillance</td>
<td>Hospital-based</td>
<td>May 1998 to May 2000</td>
<td>0 – 3 years</td>
<td>281/834 (34%)</td>
<td>11% (31/281)</td>
<td>Gabbay (personal communication)</td>
</tr>
<tr>
<td>E, Pre-trial surveillance</td>
<td>Hospital-based</td>
<td>March to October 2003</td>
<td>0 – 5 years</td>
<td>343/762 (45%)</td>
<td>68%* (232/343)</td>
<td>Abate (2004)</td>
</tr>
</tbody>
</table>

* 50 samples were selected to be analyzed.
PAGE OF G1 STRAINS

The RNA profiles were visualized in 116 (78%) of 148 G1 samples tested. Based on migration differences of gene segments 2, 5 and 10, three distinct long electropherotypes were identified (L1, L2 and L3). When the L1 electropherotype is compared with L2, gene segment 2 of L2 migrates more slowly than its corresponding gene in L1. With regards to L3, migration differences for gene segments 5 and 10 were seen with respect to L1. An additional gene segment was found in a sample displaying the L1 pattern (Figure 1). The most frequent electropherotype was L1 (79%, 92 of 116). Study E provided the highest number of samples available for further characterization by PAGE (96%, 48 of 50).

G AND P GENOTYPING

One hundred and forty seven samples bearing G1 specificity and one sample displaying a mixed G1+G4 infection were found. P type could be determined in 130 (88%) samples. P[8] was the most frequent VP4-specificity (64% of cases). In addition, the mixed infections P[6]+P[8], P[4]+P[8], P[4]+P[6] and P[4]+P[6]+P[8] were found in 11 (7%), 11 (7%), 3 (2%) and 1 (0.6%) samples, respectively. Table 2 shows PAGE and RT-PCR (VP4 and VP7 genes) characterization of the 148 samples. The G1P[8] combination displaying the L1 pattern was responsible for 45% of infections.

DISCUSSION

G1 rotavirus still seems to be the most prevalent genotype, representing approximately half of the strains circulating worldwide. In developed countries, G1 occurs at the highest frequencies (70%-73%). In developing countries, these rates appear to be lower (36%-57%). These differences might be due to the more frequent circulation of unusual strains, as well as the emergence of new types in the poorest countries.

In the present report, the G1 strain was detected in 41% of rotavirus cases over 21 years in studies using different approaches in target populations. Our results are similar to those of a study carried out in Rio de Janeiro, where 50% of samples obtained from children with acute diarrhea were G1 type. Parra et al. reported G1 strains in 17% of cases in a study conducted in Paraguay from 1998 to 2000. This difference might be associated with the high prevalence of unusual strains, as well as the emergence of new types in the Paraguayan study.

G1 rotavirus rates ranged from 11% to 68% across the analyzed studies. The lowest rates were observed between 1998 and 2000. It is likely that these results are related to the high frequency of untyped strains, as well as to the emergence of G9 during this period. Santos et al. reported that 79% of all samples analyzed in Salvador were G9. Similar results were also reported in Goiás, where Costa et al. detected 34% G9 rotavirus infections. These findings suggest that the emergence of G9 in Brazil was followed by wide circulation of strains with this genotype.

It is worth mentioning that all G1 samples displayed long electropherotypes in the present analysis. These RNA profiles showed three different patterns, thus demonstrating a broad diversity of circulating electropherotypes in the region. All varieties were found in specimens from Studies B and D, in which a high frequency of unusual and untyped strains were detected (data not shown). Luz et al. detected two varieties of long electropherotypes and 19% G1 specimens bearing a short profile with three varieties circulating among children with diarrhea in Maranhão.

The G1P[8] genotype has been found circulating in several countries as the predominant type. In the present study this genotype was detected in 64% of the samples. Similar results were obtained by Gentsch et al. and Castello et al. in studies conducted in Latin America, who
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RESUMEN


Palavras-chave: Gastroenteritis; Infecciones por Rotavirus; Variação Genética.

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RESUMEN


Palabras clave: Gastroenteritis; Infecciones por Rotavirus; Variación Genética.
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