Surveillance epidemiological: parvovirus B19 genotype 1

Vigilância epidemiológica: parvovirus B19 genótipo 1

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*****Maria Isabel de OLIVEIRA[1], Ana Maria Sardinha AFONSO[1], Cristina Adelaide FIGUEIREDO[1], Ana Lucia FRUGIS YU[2], Suely Pires CURTI[1]

*Endereço de correspondência: [1]Instituto Adolfo Lutz, Serviço de Virologia, Av. Dr. Arnaldo 355, CEP 01242-902, São Paulo,SP/ Brasil. Fone (055 11) 3068.2903 e-mail: olive40@uol.com.br
[2]GT, Exantemáticas/Ministério da Saúde/São Paulo/Centro de Vigilância Epidemiológica/DDTR, São Paulo, SP/ Brasil.
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RESUMO

O parvovirus humano B19 foi isolado e caracterizado de amostra clínica de um paciente, infectado no Japão, e que apresentou os sintomas de febre e erupção cutânea após sua chegada ao Brasil. A infecção por parvovírus foi confirmada por meio de seguintes ensaios: Elisa para detecção de anticorpos IgM anti-parvovirus B19 e técnica de *polymerase chain reaction* (PCR). Um fragmento da região NS1-VP1 foi diretamente submetido ao seqüenciamento do nucleotídeo. A análise filogenética parcial do B19, frente às várias seqüências disponíveis no *GenBank*, indicou que PV B19 isolado correspondeu ao genótipo 1.
Palavras-chave. parvovirus humano B19, epidemiologia molecular, seqüência do nucleotídeo, erythrovirus, eritema infeccioso.

ABSTRACT

Human parvovirus B19 was identified and characterized in sample collected from a patient who was infected in Japan, and the symptoms as fever and rash appeared after arriving to Brazil. The occurrence of virus infection was confirmed by both assays: Elisa parvovirus B19-specific IgM antibody detection and polymerase chain reaction (PCR). A fragment of NS1-VP1 region was directly submitted to nucleotide sequencing. Partial phylogenetic analysis of B19 sequences, including several sequences available in GenBank, indicated that the isolated HPV B19 corresponded to genotype 1**.**
Key words. human parvovirus B19, molecular epidemiology, nucleotide sequence, erythrovirus, erythema infectiosum.

INTRODUCTION

Human parvovirus B19 (HPV B19) belongs to the genus *Erythrovirus* of the *Parvoviridae* family[1]. HPV B19 infection is associated with a wide range of clinical symptoms. The most common manifestation caused by HPV B19 infection in children is an erythema infectiosum disease, which results in mild fever and rash. In adults, the clinical features vary from asymptomatic or mild to acute illnesses including acute arthritis[2]. The most commonly transmission by personal contact is via aerosol or respiratory secretions. Viraemia occurs 1 week after exposure and usually lasts about 5 days, with virus titers peaking on the first 2 days [(18)]. In general, individuals are susceptible to a certain course of infection or differences in the HPVB19 genome that can result in a more virulent strain[3,4,5].

As a result of increased awareness of the importance of screening for HPV B19, a number of novel genotypes have been identified. Genetic diversity among HPV B19 virus strains has been reported to be very low, with less than 2% of nucleotide divergence in the whole genome. Servant et al.[6] carried out a phylogenetic analysis of partial sequences, combined with the erythrovirus sequences available in GenBank, and thus proposed that the erythrovirus group should be classified into three individualized genotypes. The HPV B19 viruses correspond to genotype 1, whereas the V9 viruses are subdivided into genotypes 2 and 3[7,8]. In this study, we accounted for a sample of HPV B19 virus that was isolated from a patient, who was infected in Japan but developed symptoms after arriving in the state of Sao Paulo, Brazil. Molecular characterization of viruses and the confirmation of the source of virus infection were possible because of the effectiveness of the measles control program.

MATERIAL AND METHODS

The case was reported as measles, as suspected by health workers from the epidemiological surveillance of the

measles control program in Sao Paulo State, Brazil. A 51-year-old woman who was living in Japan arrived in the state of Sao Paulo on January 18, 2005, featuring fever (38.0°C) for 4 days, followed by the onset of a rash on January 24, 2005. An epidemiological investigation on the virus infection started on January 28, 2005 by collecting blood.

The sample was analyzed at Adolfo Lutz Institute, in the state of Sao Paulo, to detect either measles or rubella infection by employing serological and nested polymerase chain reaction (PCR). Because all tests were negative, the blood sample was then tested for HPV B19 infection, which was demonstrated to be positive. Testing for B19 virus specific antibodies was performed using a commercial IgG and IgM enzyme immunoassay kit (Biotrin®, Dublin, Ireland) that was specific for identifying VP2. The test was performed according to the manufacturer recommendations. DNA was extracted from sera with QIAmp® DNA minikit (Quiagen, Courtabocuf, France) according to the manufacturers' instructions. The oligonucleotide primers specific to a conserved region of the NS1 gene encoding for HPV B19 nonstructural protein (positions 1399 to 1659) were used for PCR diagnostic, as previously described[9]. For sequencing a region of the NS1-VP1 gene of an erythrovirus HPV B19 sample was amplified by PCR with oligonucleotides described by Servant et al.[6]. The PCR products were sequenced directly using the "ABI Prism^{R} Big Dye^{M} Terminator Cycle Sequencing Ready Reaction Kit" according to the manufacturer's protocol. Sequences were determined in an ABI sequencer model 377 (PE Applied Biosystems, Foster City, CA, USA). The phylogenetic analysis of viral strains was compared with the sequences of the GenBank (Table 1). Sequence generated from the HPV B19 sample was submitted to Blast searches in the GenBank [10], and thus aligned with prototype sequences of genotype 1, strain Pvbua, genotype 2, prototype strain Lali, and genotype 3, prototype strain V9. Sequence alignment was performed using the multiple sequence alignment method implemented in CLUSTALX[11]. Accuracy of nucleotide sequence alignment was examined using amino acid sequence alignment.

For the phylogenetic analysis we used a fragment of the genes, positions 2103 to 2626. Maximum likelihood (ML) analyses were performed using PAUP 4.0b10[12].

The best model in PAUP was chosen using ModelTest 3.06[13]. This program uses both a hierarchical Likelihood Ratio Test (hLRT) and the AIC (Akaike Information Criterion) to choose among 56 available models. Maximum likelihood searches started with a neighbor-joining tree, on which we then optimized parameters and fixed the values for those parameters for branch swapping on that tree. For each bootstrapped data set PAUP did TBR branch-swapping and only one tree was saved in each replicate. Support for each clade generated for unpartitioned data sets was assessed by 1000 bootstrap replicates using PAUP.

RESULTS

The erythrovirus HPV B19 infection was confirmed by both parvovirus B19-specific immunoglobulin M (IgM) and PCR assay. Sequencing of the sample DQ453960 generated a fragment of 524 bp (positions 2103 to 2626 in the alignment with AF162273) of the NS1 - VP1 gene, which is deposited in GenBank. Both nucleotide and amino acid sequences of DQ453960 were used for comparisons to the same region of the HPV B19 genome by a BLAST search in GenBank (Table 1) to

Table 1. Characteristics of HPV B19 virus subjected to phylogenetic analyses

Strain/year	Group	Locality	GenBa nk number
Pvbaua, 1986	1	Canadian	M13178
Pvbpro, 1987	1	Canadian	M24682
Pvb19nsvp, 1995	1	London	Z68146
Pvb19x528, 1996	1	Germany	Z70528
N8/K, 1999	1	Japan	AB030673
HV/G, 1999	1	Italy	AF162273
R43, 1999	1	France	AJ249435
V9, 1999	3	France	AX003421
R225, 2001	2	France	AJ249431
R322, 2001	2	France	AJ249434
Lali/K, 2001	2	France	AY044266
E99.2, 2002	3	France	AY083238
E99.3, 2002	2	France	AY083239
Br 543, 2004	3	Brazil	AY647977
*BrSP 1, 2005	1	Brazil	DQ453960

(*) porposed genotype

corroborate the sample identity based on specific immunoglobulin M assay.

Comparisons of the nucleotide and amino acid NS1 - VP1 gene sequences of the strain BrSP1 (DQ453960) with those from representative of the HPV B19 deposited in the GenBank showed a high level of identity. Nucleotide pairwise identity among strains ranged from 88.8% to 99.6% in genotype 1, and 91.6% to 99.4% in genotype 2, and 89.8% to 92.5% in genotype 3. Amino acid identity ranged from 95.9% to 100% in genotype 1, 97.6% in genotype 2, 96.5% to 99.4% in genotype 3 (MEGALIGN, DNASTAR, Inc.).

The alignment of a fragment of the NS1 - VP1 gene sequences, employed for the present study is available upon request. ModelTest 3.06[10] was used to choose among models in PAUP. The hLRT found the HKY + model, while the AIC suggested the TVM + model (Transversion Model with gamma distributed site-to-site rate variation). The latter model was chosen. ML analysis under the TVM + model generated a single topology with a log ML of –2535.70709. The ML topology recovered a clade consisting of all eight sequences of the genotype 1; a second clade consisting of genotype 2 sequences, AJ249431, AJ249434, AY044266, AY083239; and a third clade leading to AX003421, AY083238, AY647977 sequences of genotype 3. Sequence DQ453960 generated from the BrSP1 isolate was recovered in the clade formed by sequences representatives of the genotype 1 (Figure 1).

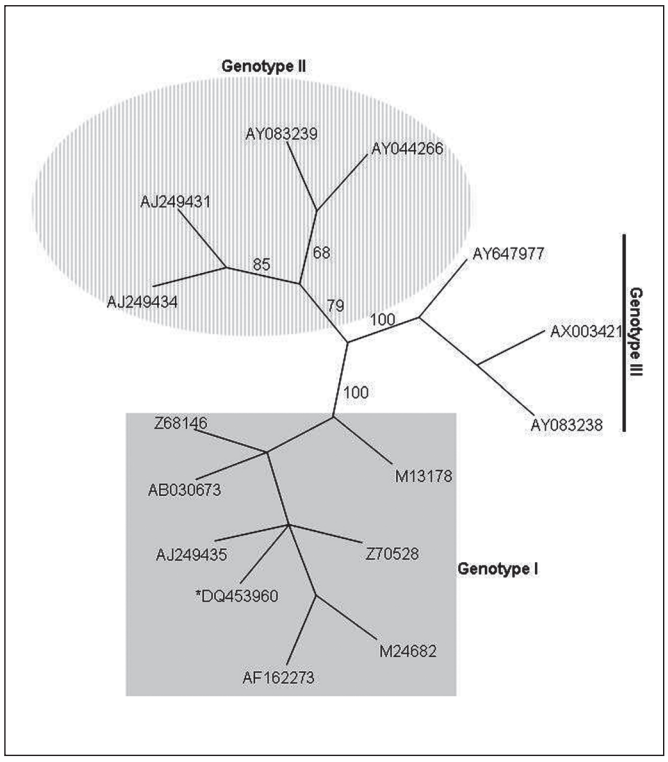

Figure 1. Phylogenetic analysis of HPV B19 based on the NS1/VP1 genes. The unrooted topology identified in the maximum likelihood analysis of 524 base pairs. Genotypes 1, 2 and 3 are labeled according to the scheme by Servant et al., 2002, Log likelihood of – 2535.70709, gamma shape = 0.198752.
(*) = the propose genotype.

DISCUSSION

Human Parvovirus B19 exanthem is highly variable, and may be misdiagnosed as rubella or measles if a laboratory investigation is not performed. Both the virological surveillance and standard epidemiological case investigation carried out by the Measles Control Program in Sao Paulo State, Brazil, confirmed that a human female was infected in Japan and traveled to Brazil during the incubation period, arriving in Sao Paulo with clinical symptoms of the disease. The patient was first investigated for both measles and rubella by specific IgM and PCR assays. The results were negative for both viruses, and thus further investigated for HPV B19, for which was positive.

HPV B19 specific IgM antibodies are detected late in the viraemic stage, about day 10 or 12, and can persist for up to 5 months[14]. For the case reported here, we detected day 10 specific IgM and the IgM was complemented with a specific diagnostic HPV B19 PCR test that improved the sensitivity of the detection of the B19 infection, just as many clinical laboratories now complement by Saldanha et al.[15]. In chronic B19 infection, DNA virus persists in the host without the presence of B19 IgM or IgG. However, it has also been shown that HPV B19 DNA can persist in healthy, immunocompetent individuals at low levels for long period of time[14,16,17].

The genetic diversity among B19 virus isolates has been reported to be very low in the whole genome, although full-length sequences are available in the GenBank only for DQ453960 number of isolates BrSP1. A PCR assay was used for screening the new isolate erythrovirus V9, but because false-negatives may be observed with respect to non-HPV B19 strains, it was necessary to use a nested PCR using separate sets of primers for both B19 and V9[18]. This nested PCR can distinguish between different gene lineages within the Erythrovirus. The evolutionary relationships among these viruses, the high ratio of synonymous to no synonymous substitutions might be indicative of an ancient separation between the B19 and V9 lineages, which accumulate with time and are not subjected to selection pressure[6,19]. Genotype 3 is markedly different from other HPV B19, 11%. The high degree of variance emphasizes the need for new diagnostic tools. Although the taxonomic grouping of V9 is unclear, and this genotype 3 is not prevalent in communities, 99% of samples were found to contain B19 DNA[18]. A molecular epidemiological investigation was carried out in Brazil to investigate which genotype was circulating in 2003 (GenBank AY647977), and

2004 (GenBank DQ229351). Comparisons of amino acid and nucleotide sequences of both samples confirmed that they belong to the genotype 3.

In contrast, to examine the epidemiological role of HPV B19 genotype 1 and 2, serological tests and PCR were conclusive in demonstrating a response characteristic of acute HPV B19 infection, because these genotypes are known to undergo to 1-2% of genetic variation in the entire genome[8,19,20]

Several studies described genotype of erythrovirus samples but none could differentiate between cases of endemic or imported HPV B19 virus. As previously mentioned, the sequence BrSP1, was compared to three different genotypes. The results of the phylogenetic analysis as well as of the sequence data analysis showed that this samples belongs to genotype 1, and that it is similar to those groups which were circulating in Japan in 1999, France 1999, 2001, 2002, Italy in 1999, Canadian in 1986, 1987, Brazil in 2004 (GenBank DQ229355), 2005 (GenBank DQ229359).

Further studies are needed to establish a new classification for the Erythrovirus gens based on genotypes and to evaluate the virological and medical significance of these genotypes. Therefore it is important to help the public health surveillance services to monitor the pathways of transmission worldwide.

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