Detection and Identification of Human Herpesvirus type 6 (HHV-6) Infection by Polymerase Chain Reactions and Restriction Endonuclease Analysis

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Abstract

Human herpesvirus 6 (HHV-6) is a widespread causative agent of exanthum subitum or roseola infantum. The species of HHV-6 is divided into two variants, HHV-6A and HHV-6-B, which might be detected and identified by PCR and RE methods. The three samples of eye swabs from children with suspected HHV-6 neurological illness were extracted by guanidium isothiocyanate method before running in PCR followed by agarose electrophoresis as a detection stage. The positive results were then identified by RE analysis to determine the variant of the viruses. One of the three samples (sample 2) shows positive result in direct electrophoresis and it was identified as HHV-6B which produced two fragments of 153bp and 70bp in the RE analysis. PCR and RE analysis are useful methods in detection as well as identification of HHV-6.

Keywords: HHV-6, PCR, Restriction endonuclease analysis

INTRODUCTION

Human herpesvirus 6 (HHV-6) is a widespread causative agent of disease in the first 2 years of life, namely exanthum subitum or roseola infantum, characterized by high fever for a few days and appereance a rash coinciding with subsidence of the fever (Yamanishi et al., 1988). HHV-6 infects almost 90% of people aged from under 10 to 59 years without showing substantial difference in the positive rate for all age group (Okuno et al., 1989). The virus was initially isolated in 1986 from peripheral blood lymphocytes (PBL) of patients with lymphoproliverative disorders and immunes abnormalities (Salahuddin et al., 1986). Electron microscope revealed HHV-6 is an enveloped virus, and icosahedral symmetry with 162 capsomers containing large double stranded DNA genome. (Salahuddin et al., 1986, Joseph et al., 1986).

HHV-6 belongs to Roseolovirus genus of the β-herpesvirus subfamily and the species of HHV-6 is divided into two variants: HHV-6 variant A and B (HHV-6A and HHV 6-B) (Ablashi et al., 1991). This division is also supported by other studies using monoclonal antibodies (Chandran et al., 1992), PCR amplification, and DNA hybridization (Aubin et al., 1990). The two variants are closely related but show consistent differences in biological, immunological, epidemiological, and molecular properties (Mori & Yamanishi, 2007).

In order to detect HHV-6 infection, there are some methods that might be approached by serological methods as well as molecular methods. Serological diagnosis is conducted by indirect immunoflourescence for the presence of antibodies to HHV-6 (Irving & Anthony, 1990). On the other hand, molecular methods for detection of HHV-6 can be conducted by PCR amplified by agarose gel electrophoresis and dot blot hybridization with a cloned DNA probe (Kondo et al., 1990).

The aim of the paper is to give a brief description of detection of HHV-6 infection and identification of its variant in the communal and clinical settings by means of PCR and restriction endonuclease typing respectively.

MATERIALS AND METHODS

DNA extraction

The extraction was conducted by guanidium isothiocyanate method. Three samples used in this experiment were eye swabs in virus transport medium from children with suspected HHV-6 neurological illness. In this stage, both infected cells and distilled water would be extracted and purified as positive and negative control. For each sample to be extracted 50μl was added into a sterile eppendorf tube containing 200 μl extraction buffer (4M guanidinium isothiocyanate, 25 mM sodium citrate, 0.5% (w/v) sarcosyl, 1mM dithiothreitol, and 62 μg/cm³ glycogen) before it was incubated at room temperature. After 10 minute incubation, 25μl 3M sodium acetate was added into a sterile eppendorf tube containing 200 μl extraction buffer (4M guanidinium isothiocyanate, 25 mM sodium citrate, 0.5% (w/v) sarcosyl, 1mM dithiothreitol, and 62 μg/cm³ glycogen) before it was incubated at room temperature.
added to each tube and mixed well. To each tube, 275µl ice-cold iso-propanol was added and mixed by inversion before it was incubated at room temperature for 5 minutes. The sample was then centrifuged at 12,000g for 10 minutes and the pellet was collected carefully. Having been added with 550µl ice-cold 70% ethanol, the sample was centrifuged at 12,000g for 10 minutes. After centrifugation, the pellet was collected carefully and allowed to air dry for 5 minutes before it was dissolved in 50µl sterile distilled water.

**PCR and agarose gel electrophoresis**

To prepare PCR reaction mixture, 50µl 10x PCR buffer (1cm3 1M tris-HCl pH 8.3; 5 cm3 1M KCl; 200 µl 1M MgCl2; 1 cm3 2% (w/v) gelatin and 2.8cm3 sterile distilled water stored at 20°C) was added into a 1.5cm3 eppendorf tube. Other components (10µl deoxynucleotides, 5µl primer H6-6, 5µl primer H6-7, 277.5µl sterile distilled water, and 2.5µl taq DNA polymerase GOLD (Roche) were then added sequentely. The tube was then mixed well and dispensed into 45µl amount in 0.2 cm3 tubes. After preparing PCR reaction mixture, 5µl of DNA sample was added to a vial of reaction mixture. Each tube and prepared extracted DNA of HHV-6A and HHV-6B as control were amplified in PCR machine (Perkin Elmer 9700) with appropriate cycling programme (94°C/7 minutes; 50°C/30 seconds; and 70°C/30 seconds for a cycle and 94°C/30 minutes; 50°C/30 seconds; and 70°C/30 seconds for 40 cycles).

The oligonucleotides used as primers or probes in PCR were designated H6-6 (5'-AAGCTTGCACAAATGCCTTTTTTGTGCAG-3' position 103133-103109 for variant A and 10426-104242 for variant B) and H6-7 (5'-CTCGAGATGGCAGACCCCTAATC3' position 102911-102935 for variant A and 104047-104131 for variant B). Amplification using primers H6-6 led to 223bp fragment, whereas primers H6-7 allowed 220bp fragment to be amplified.

Agarose gel electrophoresis was carried out by E-gel® (invitrogen) method. To a 0.5ml eppendorf tube containing 10µl sample, 1 µl loading buffer (10mM tris-HCl pH 7.5; 1mM EDTA; 0.005% bromophenol blue) was added followed by adding 10µl sterile distilled water. Onto a 2% E-gel, 20µl of each sample was loaded and 10µl DNA molecular weight marker (invitrogen) was loaded as well. To all unused wells, 20µl sterile distilled water was loaded before the gel was run for 15-30 minutes and visualised on a transluminator so that variant of HHV-6 product might be determined.

**RESULTS**

**Detection of HHV-6 infection in the samples**

In this experiment, both extract positive and negative control were extracted and purified as described above in order to ensure that there is no distortion during amplification in PCR. DNA of HHV-6A and HHV-6B was also loaded to ensure the specificity of the added primer in amplification.

From three samples assayed by PCR and direct electrophoresis, sample 2 shows the positive result of HHV-6 infection due to the same fragment as extract positive control, and both extracted DNA of HHV-6A and HHV-6B accounting for 233bp (Figure 1).

**Restriction endonuclease typing**

In 0.5ml eppendorf tubes, three sets of digestion mixtures were prepared. The first set contained 5µl amplicon, 2µl RE buffer A, 1µl AvaII (Roche), and 12µl sterile distilled water. The second set contained 5µl amplicon, 2µl RE buffer H, 1µl HindIII (Roche), and 12µl sterile distilled water, whereas the final set contained 5µl amplicon, 2µl RE buffer A, 2µl RE buffer H, and 13µl sterile distilled water. All tubes were then incubated at 37°C overnight. After incubation, 2µl loading buffer was added to all tubes before 10µl sample was loaded onto a 10cm3 of 8% polyacrylamide gel (6.9 cm3 sterile distilled water, 2 cm3 acrylamide:bis acrylamide (37:5:1), 1 cm3 tris-borate-EDTA buffer, 9µl TEMED, and 100µl 10%(w/v) ammonium persulphate). To one well, 10µl Hyperladder II (Bioline) as DNA molecular weight marker was also loaded. After loading samples and marker, the electrophoresis was carried out at 100V for 45 minute before it was then stained with ethium bromide and visualised on a transluminator so that variant of HHV-6 product might be determined.

**Fig 1.** Separation of viral DNA by PCR and direct electrophoresis gel (left) and the DNA molecular weight markers (right). A specifically-amplified band for HHV-6 corresponds to 233 basepairs.

**Restriction endonuclease typing**

In determination of variants of HHV-6 product, HHV-6 variant A is cleaved by AvaII producing two fragments of 141 bp and 82 bp in the electrophoresis gel, and HHV-6 variant B
cleaved by \textit{Hinfl} produces two fragments: 153 bp and 70 bp.
Although HHV-6A and HHV-6B are closely related, these two variants are consistently different in biological and epidemiological features. The genome of HHV-6A is 159,321 bp (Gompels et al., 1995) as opposed to HHV-6B which is 161,144bp (Domínguez et al., 1999). In terms of replication in specific transformed T-lymphocyte cell lines, HHV-6A propagates in the T-cell line HSB-2, whereas HHV-6B grows in primary lymphocytes and in the Molt-3 or MT-4-T cells line (Mori & Yamanishi, 2007). Furthermore, there is no clear disease has yet been associated with HHV-6A, but HHV-6B is obviously the major causative agent of exanthem subitum (ES) (Yamanishi et al., 1988).

PCR assay and restriction enzyme analysis are useful methods in detection as well as identification HHV-6 in terms of their sensitivity and specificity. PCR assay might be helpful, particularly in the development of HHV-6 drugs such as acyclovir and ganciclovir, which are available for the treatment of another viruses, namely HSV, VZV, and CMV infection (Kondo et al., 1990). Restriction endonuclease analysis, on the other hand, might be also helpful in determination of genetic polymorphism affecting the pathogenicity and biological properties as well as viral transmission between two strains of the same HHV-6 species (Aubin et al., 1991).

**REFERENCES**


