Detection of cytomegalovirus (CMV) DNA by PCR in patients with unknown inflammatory eye diseases

ABSTRACT

Cytomegalovirus (CMV) is well known as a pathogenic agent of intraocular inflammatory diseases. Light microscopy and immunohistochemical studies have limitations in the identification of this virus, but detection and typing viral DNA with Polymerase Chain Reaction (PCR) offers a rapid, highly specific, and easily interpretable means of identifying CMV in patients with ophthalmic lesions. Two patients (34-year-old male and 48-year-old male) who developed retinitis with unknown causative agent were studied for presence of CMV DNA. We used PCR kit for the qualitative detection of Cytomegalovirus (CMV 500/800 IC) provided for us by “Sacace Biotechnologies”*. The target of the PCR reaction was the “Major Immediate-Early” (MIE) gene. Positive and negative controls were used to avoid false results. Our PCR analysis showed the presence of CMV-DNA within the samples.

Key words: Cytomegalovirus, CMV, DNA, PCR, inflammatory eye disease

Introduction

Intraocular inflammation caused by viral infection is one of the major known types of endogenous inflammatory eye diseases (Koizumi et al., 2008). Herpesviruses are common pathogens of retinitis but uncertain identification of herpesvirus primary infection and reactivation symptoms can make diagnosis difficult (van Boxtel et al., 2007). CMV rarely causes retinitis in non-immunocompromised adults but is typical in congenitally infected neonates (Usui et al., 1993). It is well known that a number of ocular surface diseases such as papillary conjunctivitis, chronic blepharitis, or dry eye are associated with infections by this family of viruses due to their ubiquitous nature (Ergazaki et al., 1994; Karavellas et al., 2001; Martin et al., 2002; Eid et al., 2008). In the present study, we employed PCR method to detect viral DNA in aqueous humour from two patients with clinically diagnosed retinitis.

Materials and Methods

DNA extraction

DNA-Sorb-B Kit (Sacace Biotechnologies) was used for total DNA isolation. We used the protocol supplied by the manufacturer. 100 µl of each sample, 10 µl if Internal Control and 300 µl of Lysis Solution were mixed together in appropriate tubes. The tubes were incubated for 5 min at 65°C then centrifuged briefly for 7-10 sec. 20 µl of DNA Sorbent was added to each tube followed by incubation for 6 min at room temperature. Then all tubes were centrifuged for 30 sec at 5000g. The supernatant from each tube was removed by a micropipette without disturbing the pellet. 300 µl of Washing Solution 1 were added to each tube followed by centrifuging for 30 sec at 8000g. The supernatant was removed and discarded. 500 µl of Washing Solution 2 were added to each tube this time. The supernatant was removed again. This step was repeated and then the tubes were incubated with open cap for 5 min at 65°C. The pellet was resuspended in 50 µl of DNA-eluent and incubated for 5 min at 65°C. The tubes were centrifuged for 1 min at 12000g and the DNA in supernatant was ready to be used for PCR amplification.

Control DNA

The positive and negative controls used in this study were supplied in the CMV 500/800 IC Kit.
PCR amplification

CMV 500/800 IC Kit (Sacace Biotechnologies) was used for PCR amplification. 10 µl of PCR-mix-2 and 10 µl of DNA of each sample were added to appropriate ready-to-go PCR-mix-1 tubes. 10 µl of DNA-buffer were added to the tube for Negative Control and 10 µl of Positive Control were added to the tube for Positive Control Amplification. On the thermalcycler (iCycler, BIORAD) the following program was performed:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Duration</th>
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<tr>
<td>95°C for 5 min</td>
<td>42 Cycles</td>
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<tr>
<td>95°C for 10 sec</td>
<td></td>
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<tr>
<td>65°C for 25 sec</td>
<td></td>
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<tr>
<td>72°C for 10 sec</td>
<td></td>
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<tr>
<td>72°C for 1 min</td>
<td></td>
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<tr>
<td>4°C – Storage</td>
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Analysis of PCR results was based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments was CMV (MIE gene) – 500bp, Internal Control – 723 bp.

Results

PCR amplification revealed CMV DNA in the studied samples (Figure 1). The Internal Control was amplified as opposed to the Negative Control. This assured us that the results were valid.

Table 1. 1 – DNA ladder (100bp); 2 – Positive control; 3 – Negative control; 4,5 – Samples. 500 bp – MIE gene product, 723 bp – Internal control product

Discussion

Intraocular inflammation caused by viral infection represents one of the major known types of endogenous inflammatory eye diseases. Infections caused by herpesviruses are very common in immunosuppressed and immunocompromised patients (Ergazaki et al., 1994; Musch et al., 1997; Karavellas et al., 2001; van Boxtel et al., 2007). Moreover, ocular diseases in these specific populations are strongly associated with infections caused by members of the herpesvirus family. Although a clinical diagnosis of viral ocular infection is most frequently made in patients with atypical features, it may be difficult to differentiate, based on clinical findings alone, between viral infections due to herpes group viruses or other nonviral pathogens (Eid et al., 2008; Koizumi et al., 2008). Cell culture of intraocular specimens testing for cytopathic effect (CPE) is the standard method for making a specific virologic diagnosis of inflammatory intraocular disease. However, it appears to be particularly difficult when only a very small quantity of intraocular fluid is available. Moreover, cell culture is both expensive and time-consuming. That is why the use of specific and sensitive technique such as PCR together with standard methods will improve greatly the management of such ocular viral infections.

References