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PCR-based diagnosis of adenovirus and Epstein–Barr virus in paraffin-embedded heart tissue

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Abstract. Immunohistochemical and molecular-pathological techniques have improved the diagnosis, but the incidence of virus-induced lethal myocarditis still remains unclear. Therefore, it is of great interest to investigate postmortem myocardial samples in cases of sudden infant death syndrome (SIDS). Adenoviruses and Epstein–Barr viruses are known as possible agents of myocarditis. Viral DNAs were specifically isolated and amplified from formaldehyde-fixed material. At autopsy, myocardial samples were taken from 62 SIDS cases from different regions and investigated with PCR. Adenoviral DNA was detected in 2/62 and Epstein–Barr viruses in 3/62 cases. Our results emphasize the importance of modern molecular-pathological methods in cases of sudden unexpected death.

Keywords: Adenoviruses; Epstein–Barr virus; PCR; Paraffin-embedded tissue; Myocarditis

1. Introduction

Studies of myocarditis in adults demonstrated that numerous cases of acute myocarditis can not be diagnosed, according to the Dallas criteria [1], by traditional hematoxylin–eosin staining of endomyocardial biopsies. Previously, we reported on detection of enteroviruses (EV) including coxsackieviruses B3 (CVB3) and parvovirus B19 (PVB19) [2–5]. We analysed adenovirus (AV) DNA and Epstein–Barr virus (EBV) DNA from paraffin-embedded heart tissue with PCR. Therefore, we established a reliable method to isolate DNA from formaldehyde-fixed and paraffin-embedded material.

2. Materials and methods

Postmortem myocardial samples were obtained from 62 autopsy cases with suspected sudden infant death syndrome (SIDS). Eight myocardial samples were taken from each heart at standardized locations. Viral DNA was extracted from paraffin-embedded myocardial, liver and spleen samples with the Genial First-DNA-Kit (Genial, Troisdorf, Germany). The prerequisite for virus PCR was the amplification of cyclophilin (cyc) [6]. To avoid false-positive results due to contamination, negative controls were performed in

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Table 1
Details of PCR primers used to amplify viral genes; AV(3); EBV(29); cyc(9)

Primer	Nucleotide sequences (5′ to 3′)	Product size (bp)
AVF	GCC GCA GTG GTC TTA CAT GCA CAT	300 bp
AVR	CAG CAC GCC GCG GAT GTC AAA GT	(AVF/AVR; first round)
AV3	AGA CGT ACT TCA GCC TGA A	130 bp
AV4	CCT TGT ACG AGT ACG CAG TA	(AV3/AV4; second round)
EBV1	AAG GAG GGT GGT TTG GAA AG	309 bp
EBV2	AAC AGA CAA TGG ACT CCC TTA	(EBV1/EBV2; first round)
EBV3	ATC GTG GTC AAG GAG GTT CC	208 bp
EBV4	ACT CAA TGG TGT AAG ACG AC	(EBV3/EBV4; second round)
CycF	CGTC CAG CAT TTG CCA TGG A	180 bp
cycR	GAC AAG GTC CCA AAG ACA G	

Table 2
Details of PCR conditions used to amplify viral genes; AV [9]; EBV [12]; cyc [6]

Primer	Denaturation (°C)	Annealing (°C)	Extension (°C)	Number of cycles
AVF	94 (60 s)	58 (60 s)	72 (60 s)	30 (first round)
AVR				20 (second round)
AV3				
AV4				
EBV1	94 (60 s)	58 (60 s)	72 (60 s)	30 (first round)
EBV2				20 (second round)
EBV3				
EBV4				
CycF	94 (60 s)	56 (60 s)	72 (60 s)	55
CycR				

all experiments [7]. For each assay, positive controls were added. For details of PCR, see Tables 1 and 2. PCR products were sequenced on an ABI 310 sequencer. Sequence comparison was performed by BLAST search of NCBI Gen-Bank. PCR products were also analysed on polyacrylamide gels (Fig. 1).

3. Results

Adenoviral DNA was detected in 2 out of 62 cases of suspected SIDS, whereas 3 out of these 62 cases were positive for EBV DNA. In all SIDS cases, the myocardial samples revealed no signs of myocarditis according to the Dallas criteria using conventional histologic stainings.

4. Discussion

Acute myocarditis can be diagnosed by PCR as a rapid method [8]. Given the fact that in endomyocardial biopsies, the detection of adenoviruses and EBV would be regarded as a pathological finding [9], this can be regarded as the cause of death in cases of SIDS. In our study, the myocardial tissues had been fixed in neutral buffered formaldehyde (pH 7.4) or Notox (Earth Safe Industries) for a maximum of 48 h. This

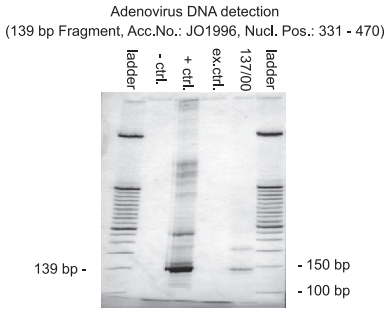

Fig. 1. Adenovirus DNA detection.

was crucial for the extraction of intact nucleic acids. After longer fixation times, reproducible PCR for viruses was not possible. The ability to isolate intact viral DNA and RNA from paraffin-embedded tissue permits the analysis of several cardiotropic viruses. Although the pathomechanisms of virus-induced myocarditis are still a matter of great scientific interest, in clinical practice it is a widely recognized fact that EBV is without any doubt cardiopathogenic when detected in endomyocardial biopsy samples [10]. Recently, a cDNA clone that encodes the common coxsackie- and adenovirus receptor (CAR) was discovered [11]. Intriguingly, this receptor is downregulated after birth and is not detectable in adults. This observation offers a molecular basis for the explanation for the cardiopathogenity of the abovementioned viruses. Our results emphasize the importance of the PCR-based diagnosis in cases of SIDS.

References

[1] H.T. Aretz, Myocarditis—the Dallas criteria, Human Pathol. 18 (1987) 619–624.
[2] A. Baasner, R. Dettmeyer, M. Graebe, B. Madea, PCR-based diagnosis of enterovirus and parvovirus B19 in paraffin-embedded heart tissue, Int. Congr. Ser. 1239 (2003) 715–717.
[3] R. Dettmeyer, R. Kandolf, P. Schmidt, M. Schlamann, B. Madea, Lympho-monocytic enteroviral myocarditis: traditional, immunohistological and molecular pathological methods for diagnosis in a case of suspected sudden infant death syndrome (SIDS), For. Sci. Int. 119 (2001) 141–144.
[4] R. Dettmeyer, et al., Fatal parvovirus B19 myocarditis in an 8-year-old boy, J. For. Sci. 48 (2003) 183–186.
[5] R. Dettmeyer, et al., Coxsackie B3 myocarditis in 4 cases of suspected sudden infant death syndrome: diagnosis by immunohistochemical and molecular-pathologic investigations, Pathol. Res. Pract. 198 (2002) 689–696.
[6] J. Ben-Ezra, et al., Effect of fixation on the amplification of nucleic acids from paraffin-embedded material by the polymerase chain reaction, J. Histochem. Cytochem. 39 (1991) 351–354.
[7] S. Kwok, R. Higuchi, Avoiding false positives with PCR, Nature 339 (1989) 237–238.
[8] A.B. Martin, et al., Acute myocarditis: rapid diagnosis by PCR in children, Circulation 90 (1994) 330–339.
[9] G.M. Lozinski, et al., Adenovirus myocarditis: retrospective diagnosis by gene amplification from formalin-fixed, paraffin-embedded tissues, Human Pathol. 25 (1994) 831–834.
[10] M.M. Hebert, et al., Fatal Epstein–Barr virus myocarditis in a child with repetitive myocarditis, Pediatr. Pathol. Lab. Med. 15 (1995) 805–812.
[11] J.M. Bergelson, J.A. Cunningham, G. Droguett, et al., Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5, Science 275 (1997) 1320–1323.
[12] K.L. Chang, et al., EBNA-1 gene sequences in Brazilian and American patients with Hodgkin's disease, Blood 94 (1999) 244–250.