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Original Communication

Postmortem diagnosis of cytomegalovirus and accompanying other infection agents by real-time PCR in cases of sudden unexpected death in infancy (SUDI)

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ABSTRACT

As an opportunistic pathogen with high mortality rates, Cytomegalovirus (CMV) may lead to fatal disseminated CMV infection of the premature and newborn; thus necessitating the demonstration of CMV-DNA with clinical history and/or histopathological findings of CMV infection and defining other bacterial and viral infection agents with real-time polymerase chain reaction (RT-PCR) in udden unexpected death in infancy (SUDI) cases as we aimed in this study.

314 (144 female, 170 male) SUDI cases were prospectively investigated from January 2013 to January 2015 in Istanbul Forensic Medicine Institution. The study includes 87 tissue samples of 39 cases for postmortem histopathological examination of interstitial pneumonia, myocarditis, meningitis, encephalitis, hepatitis, colitis or tubulointerstitial nephritis and/or accompanying chronic sialadenitis.

CMV-DNA was found positive in 35 (40.2%) salivary gland, 19 (21.8%) lung, 1 (1.1%) tonsil, and 1 (1.1%) brain tissues. CMV sialadenitis and/or CMV pneumonia associated with other viral and/or bacterial agents were detected in 23 (60%) of 39 infant cases.

The demonstration of CMV-DNA would significantly clarify the cause of death and collection of epidemiological data in SUDI cases with clinical history and histopathological findings of CMV infection accompanying chronic CMV sialadenitis. Furthermore, CMV suppresses the immune system, and may predispose to other bacterial and/or viral infections in these cases. Post-mortem molecular investigations are useful in explaining cause of death in SUDI with a suspicion of infection in forensic autopsies.

1. Introduction

Cytomegalovirus (CMV), a member of the betaherpesvirinae subfamily, is an opportunistic pathogen that remains latent in lymphocytes, polymorphonuclear leukocytes, renal epithelium cells, salivary glands and can be reactivated in case of immunosuppression.[1,2] CMV may result in asymptomatic infections in children and adults, and it may particularly cause infections with high mortality in immunocompromised individuals such as premature and neonatal infants. CMV may be transmitted through aspiration of mother's blood or infected genital secretion during birth or breast feeding and it generally remains latent in the infant.[2–4] Disseminated CMV infections (pneumonia, myocarditis, colitis, tubulointerstitial nephritis, meningitis, etc.) may have a fatal progress in infants infected with this virus, accompanying chronic sialadenitis, which declines with age.[5] Furthermore, CMV may also suppress the infant's immune system, thereby it may predispose the immune system to infections with other bacteria and viruses.[1,5]

Demonstration of histopathologically-described CMV-DNA in infants with CMV infections and/or accompanying chronic sialadenitis, and definition of the other bacterial and viral infection agents in sudden unexpected death in infancy (SUDI) cases referred to the Council of Forensic Medicine of Istanbul for autopsy were the objectives of the study herein.

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2. Materials and methods

2.1. SUDI cases

314 (144 female, 170 male) SUDI cases were prospectively investigated from January 2013 to January 2015 in Istanbul Forensic Medicine Institution. Samples were collected and histopathological examination was carried out with the routine hematoxyline and eosin staining method. The blood, CSF, lung, spleen, stool and tracheal swab samples were collected for microbiological examination. All of the cases were selected by the permission of Council of Forensic Medicine Istanbul Scientific Committee.

2.2. Bacterial cultures

Samples collected for microbiological examination were cultivated in appropriate media for aerobic and anaerobic bacteriological cultures. The identification of pathogenic bacteria was performed using conventional methods and a miniAPI (Biomerieux, France) semi-automated identification system.[6]

2.3. Detection of viruses

The multiplex PCR method was applied in order to designate the viral gastroenteritis agents (Adenovirus, Rotavirus, Astrovirus, Norovirus G1 and G2) in stool samples, and respiratory tract agents [Rhinovirus, Parainfluenzavirus (1,2,3,4), Influenza A and B Virus, Enterovirus, Human Bocavirus, Adenovirus, H1N1 Virus, Coronavirus (229,63,HKU,43), Human Metapneumovirus A/B, Parechovirus, Respiratory Syncytial Virus (RSV) A/B, *Mycoplasma pneumoniae*] in tracheal swab sample. The nucleic acids were extracted in the QIAsymphony device using a QIAsymphony DSP Virus/Pathogen midi kit, and were amplified in the Rotor GeneQ device (Qiagen, Germany) via the RT-PCR method using FTD Viral Gastroenteritis (Fast-track Diagnostics, Luxemburg) and FTD Respiratory 21 (Fast-track Diagnostics, Luxemburg) kits in accordance with the recommendations of the manufacturing company.

Viruses known as the causes of myocarditis (Enterovirus, Ebstein Barr Virus, CMV, Adenovirus, Parvovirus B19) were investigated in the tissue samples which were histopathologically considered to have myocarditis. The DNA isolation was carried out in the QIAsymphony device using a QIAsymphony DSP Virus/Pathogen midi kit, and the amplification procedures were performed in the Rotor GeneQ device (Qiagen, Germany) using an Artus (Qiagen, Germany) kit through the RT-PCR method in accordance with the recommendations of the manufacturing company.[2,3]

2.4. Histopathological examination

Amongst the cases identified to have an 'owl's eye' appearance (Fig. 1) in the post-mortem histopathological examination of salivary glands, 87 tissue samples (37 salivary gland, 38 lung, 1 tonsil, 3 kidney, 3 myocardium, 1 brain, 1 liver, 3 bowel samples) from 39 cases demonstrating findings of interstitial pneumonia, myocarditis, meningitis, encephalitis, hepatitis, colitis or tubulointerstitial nephritis (Figs. 2–4) and/or accompanying chronic sialadenitis were included in the study.[2]

2.5. Detection of CMV-DNA

DNA was extracted from all 87 formalin-fixed paraffin-embedded tissues. Three or four 10-μm-thick sections from each block were cut by means of a microtome. Paraffin-embedded tissues underwent xylene and ethyl alcohol deparafinization

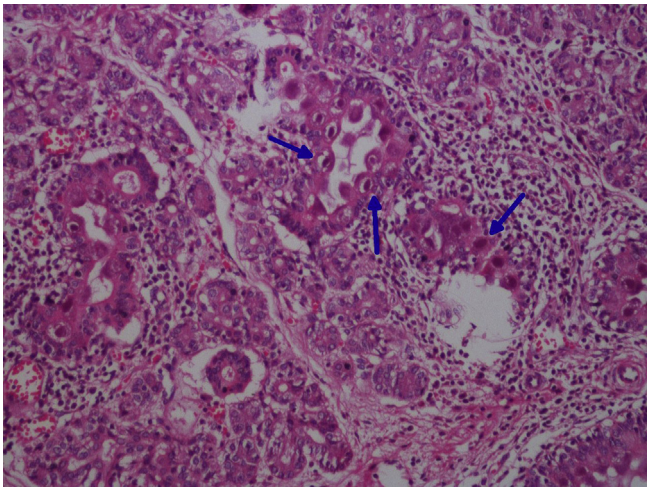

Fig. 1. Intranuclear basophilic inclusions (owl's eye) in the ductal epithelial cells and interstitial mononuclear inflammatory infiltrate on the salivary gland (H&EX200).

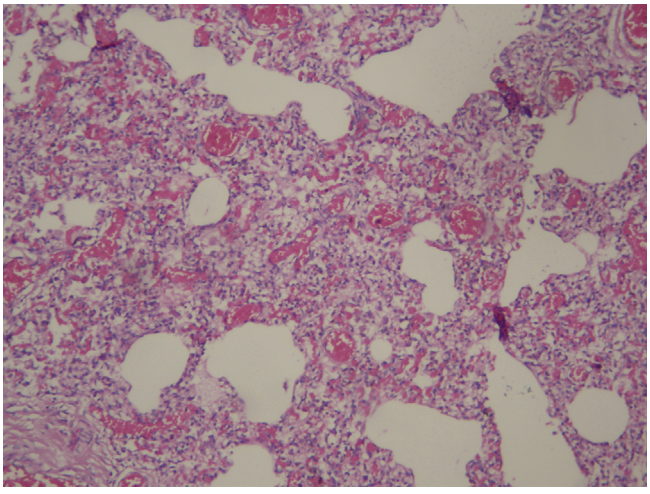

Fig. 2. Interstitial pneumonia with prominent septal mononuclear inflammatory infiltrate (H&EX200).

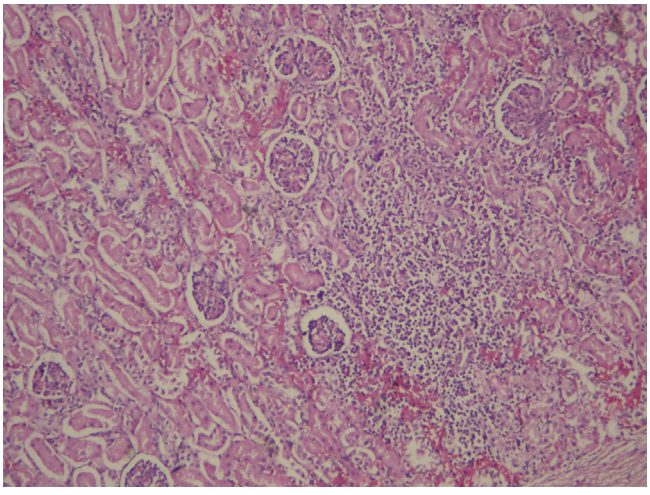

Fig. 3. Prominent mononuclear inflammatory infiltrate in the interstitium of kidney (H&EX200).