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Profiling the approach to the investigation of viral infections in cases of sudden unexpected death in infancy in the Western Cape Province, South Africa

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ABSTRACT

Background: Sudden unexpected death in infancy is one of the main contributory factors to high infant mortality rates world-wide. Several risk factors, including viral infection, have been implicated in SUDI cases, but no single factor has been confirmed as the main cause of death. At the Tygerberg Medico-legal Laboratory, Cape Town, South Africa, investigation of lung tissue for viral infection forms part of an institutional protocol for the examination of cases of sudden unexpected death in infancy.
Methods: Lung tissue from 82 cases of sudden unexpected death in infancy was collected over a 10 month period. Routine shell vial cultures and histological examination of the tissue were performed according to the standard institutional protocol on fresh and formalin-fixed tissue, respectively. In addition, real-time polymerase chain reactions and immunohistochemical staining for adenovirus, cytomegalovirus and respiratory syncytial virus were done on fresh and formalin-fixed lung tissue, respectively.
Results: Huge variation was found in the number of positive cases confirmed by shell vial culture, real-time polymerase chain reaction and immunohistochemistry (0, 2 and 0 for adenovirus; 3, 29 and 2 for cytomegalovirus; and 0, 0 and 4 for respiratory syncytial virus, respectively).
Conclusions: In the absence of a National Protocol for investigation of sudden unexpected death in infancy, we conclude that the selection of viruses and routine diagnostic technique included in the institutional investigation protocol might be suboptimal and should be re-evaluated.

1. Introduction

Sudden unexpected death in infancy (SUDI) is the collective term for cases where an infant younger than one year dies suddenly and unexpectedly [1,2]. Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant under one year of age, which remains unexplained after a thorough case investigation, complete autopsy, examination of the death scene and a review of the clinical history [3–10]. SIDS is therefore a diagnosis of exclusion after eliminating all other causes and its validity depends upon the accuracy and completeness of the investigations. In developed countries, SIDS is the leading cause of death in infants [7].

Worldwide, children under 5 years of age are extremely vulnerable to acute respiratory infections (ARI) and have on average between 6 and 8 episodes per year. It is the third most common cause of death in this age-group in Sub-Sahara Africa and accounts for almost 8% of all deaths in infants and children in South Africa [11]. The prognosis is much worse in rural Africa, where many people suffer from poor health, malnutrition, anaemia and other co-morbidities, such as malaria and human immunodeficiency virus (HIV) infection [12].

The South African Inquests Act (58 of 1959) mandates a medico-legal autopsy in all cases where the cause of death is not evident, including all SUDI cases. However, no nationally standardised death investigation protocol for SUDI exists in South Africa and all institutions follow different protocols [13]. At the time of this study, routine investigation for viral infection in cases referred to

Abbreviations: AdV, adenovirus; ARI, acute respiratory infection; CMV, cytomegalovirus; HIV, human immunodeficiency virus; IHC, immunohistochemistry; IP, interstitial pneumonitis; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; SANAS, South African National Accreditation Services; SIDS, sudden infant death syndrome; SUDI, sudden unexpected death in infancy; SVC, shell vial culture.

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the Tygerberg Medico-legal Laboratory in the Western Cape Province was limited to only adenovirus (AdV), cytomegalovirus (CMV) and respiratory syncytial virus (RSV). This was done according to a qualitative South African National Accreditation Services (SANAS) accredited shell vial culture (SVC) technique.

This study was preceded by a retrospective audit of all SUDI cases admitted to the Tygerberg Medico-legal Laboratory over a period of three years (2004–2006) and one or more of these viruses could only be confirmed in 14% of the cases (data not shown). Since no other cause of death could be found in the remaining 86% of cases, a final Cause of Death Classification of SIDS was assigned to these cases.

This study aimed to compare the results obtained from the standard SVC diagnostic technique with other methods, such as immunohistochemistry (IHC) and real-time polymerase chain reaction (PCR), to evaluate the detection rate of AdV, CMV and RSV infection in infants who succumbed to SUDI. This information is important against the background of the burden of disease in South African infants, but also has epidemiological value in identifying possible pathogens.

2. Methods

A retrospective data audit of all the SUDI case files from 2004 to 2006 was conducted to summarise routine virological laboratory results in these cases. This was followed by a prospective analysis of the lower lobe of the left lung from 82 autopsy cases admitted to the Tygerberg Medico-legal Laboratory as SUDI cases between 29 September 2009 and 27 May 2010. The project was approved by the Health Research Ethics Committee of Stellenbosch University and a waiver of consent was granted, because the study objectives did not deviate from the standard diagnostic investigation protocol at this institution.

2.1. SVC

Human fibroblasts (HF) were used for CMV and combination (combi) cultures containing both HEp2 and Madin-Darby Canine Kidney (MDCK) cells for AdV and RSV isolation from homogenised lung tissue. After incubation for 48 h, indirect fluorescent antibody (IFA) staining was done with a LIGHT DIAGNOSTICS™ Respiratory Panel 1 Viral Screening & Identification IFA Kit (Millipore, USA), according to manufacturer's instructions.

2.2. Qualitative real-time PCR

Nucleic acids were extracted from fresh lung tissue using the QIAamp® DNA Mini Kit and RNeasy® Mini Kit (Qiagen, Germany) for DNA and RNA, respectively, according to the manufacturer's instructions. Published primer and probe sequences were used for real-time PCR assays for AdV [14], CMV [15] and RSV [16]. All primers and probes were obtained from Applied Biosystems® (Johannesburg, South Africa).

2.3. Histology and IHC

Lung sections were routinely stained with haematoxylin and eosin (H&E) and IHC staining was done on the Leica BOND-MAX™ automated system using a Bond™ Polymer Refine Detection System (Leica Microsystems, Randburg, South Africa). Excess formalin pigment caused by over-fixation was removed with picric acid treatment. Specific antibodies for AdV, CMV and RSV were used in a 1:50 dilution with an optimised heat-induced epitope retrieval protocol of 40 min @ 97 °C [17]. All lung sections were microscopically evaluated and micromorphology noted with special attention to the presence or absence of pulmonary inflammation. Interstitial pneumonitis (IP) was graded according to the system devised by Krous et al. [18].

2.4. Data analysis

If grade 2 or 3 IP was noted in the absence of any other positive finding, pneumonitis was considered the most likely cause of death. Positivity on any of the three diagnostic methods was considered a positive viral infection. The results for these two parameters were cross-tabulated and correlated using the Pearson Chi-square and Fisher Exact two-tailed statistics. Statistical significance was associated with $p < 0.05$.

Kappa statistics (SAS version 9.1) was used to calculate the amount of agreement between the three diagnostic tests and interpreted as follows: Kappa Coefficient = 1 (absolute agreement) or 0 (no agreement). A Pr > S value smaller than 0.05 was considered significant.

3. Results

3.1. Epidemiological data

A total of 48 male and 34 female cases were included in the study. Their average age was 11.6 weeks (range 0.5–56 weeks) and the average interval between death and autopsy was 3.5 days (range 0–8 days). The majority of cases were black ($n = 54$, 66%), followed by mixed ancestry ($n = 27$, 33%) and only 1 was Caucasian (1%).

The environmental or risk factor reported most frequently was bed-sharing (65%), followed by minor clinical symptoms prior to death and smoke by parents (29% each), prematurity (27%), alcohol use by parents and sleeping in the prone position (24% each).

3.2. Laboratory results

Results are summarised in Table 1. Positive results for single viruses were obtained in 31 cases and only 2 cases yielded positive results for both CMV and RSV.

3.3. Histology

For the purpose of this study, grade 2 or 3 IP was regarded as possibly severe enough to cause death [18]. Significant (grade 2 or 3) IP was present in 13 (16%) of cases. However, only 6 of these had positive results for CMV alone, one was positive for RSV and another one for both CMV and RSV.

When the presence of significant IP combined with either positive results for one of the viruses or clinical symptoms before death, no combination with AdV was found. Five cases had positive results for CMV alone and 1 case for RSV alone. Three cases had clinical symptoms before death without any positive viral results and a further 2 had clinical symptoms before death and CMV infection. A single case had clinical symptoms before death, as well as positive results for CMV and RSV.

Table 1
Number of positive tests for the different diagnostic methods.

	SVC	Real-time PCR	IHC
Adenovirus	0	2 (2%)	0
Cytomegalovirus	3 (4%)	29 (35%)	2 (2%)
Respiratory syncytial virus	0	0	4 (5%)

SVC = shell vial culture; PCR = polymerase chain reaction; IHC = immunohistochemistry