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Evaluation of postmortem bacterial culture results: A retrospective study

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Postmortem blood, cerebrospinal fluid (CSF) and various clinical samples are sent to the medical microbiology laboratory for bacteriological examination in order to determine the cause of death. This study aimed to investigate the results of postmortem cultures sent to our laboratory from different clinics between January 2005 and August 2011. The culture results were retrospectively analyzed from the laboratory records. Various clinical samples obtained from 94 subjects for bacteriological examination between the above-mentioned dates were sent to the laboratory. Of the cases, 89 were from the pediatrics and the pediatric emergency department, and five were from other clinics. A single sample was sent from 26 cases, and two or more samples were sent from 68 cases for bacteriological examination. Bacterial growth was reported in 21 (35%) out of 60 blood cultures, 6 (10.3%) out of 58 CSF samples, 9 (18.8%) out of 48 urine samples, 29 (80.6%) out of 36 tracheal aspirates and 1 (16.7%) out of six the other samples. Bacterial growth was reported to be at a higher extent in the tracheal aspirate and the blood samples among the cultures. While *Candida* species and *Pseudomonas aeruginosa* were isolated most in the tracheal aspirate samples, *coagulase negative staphylococcus* and *Klebsiella pneumoniae* were isolated from blood cultures.

Key words: Forensic microbiology, postmortem, bacteriology.

INTRODUCTION

Postmortem bacteriological examinations are of importance in providing information about whether the deaths occur during the hospital stay or if forensic cases are caused by a bacteriological infection or not. Various postmortem clinical samples are sent to the microbiology laboratory in order to understand the pathogenesis of infectious diseases as the cause of death. In autopsies and postmortem analyses, examination of the biological materials obtained for determining the cause of death in terms of bacterial, viral, fungal and parasitic agents requires meticulousness and experience. Particularly in babies, microbiological methods may be decisive in sudden deaths, in cases in which other examination findings are not explanatory alone and in biological crimes (Tsokos and Püschel, 2001; Morris et al., 2006; Schutzer et al., 2005; Hove and Pencil,1998). Furthermore, postmortem bacteriological examinations are also recommended for verification of antemortem diagnosis, investigation of the etiology of the disease and assessment of treatment (Roberts, 1998). There are studies available indicating that bacteriological examination of various clinical samples is important for postmortem assessment (Lobmaier et al., 2009; Srifeungfung et al., 2005; Saegeman et al, 2009).

Although the clinical value of postmortem cultures is controversial due to postmortem contamination and the

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likelihood of postmortem spread of microorganisms, bacteriological examination of the postmortem microbiological samples is recommended, particularly in sudden unexpected death in infancy/sudden infant death syndrome (SUDI/SIDS) (Weber et al., 2008, 2010; Prtak et al., 2010).

In this study, the culture results of postmortem samples sent to our laboratory were analyzed retrospectively. The purpose of this study was to determine the frequency of pathogenic microorganisms in postmortem cultures and growth on the cultures was whether single type or polymicrobial. On the other hand, to draw attention to postmortem bacteriological examinations and their importance.

MATERIALS AND METHODS

The culture results of the postmortem samples sent to our laboratory from different clinics between January 2005 and August 2011 were evalauted. The culture results were analyzed retrospectively from the laboratory records. SPSS (Ver 16.0, USA) was used for frequency analysis of data. The culture samples which were sent after autopsy following the forensic examination were excluded. The blood samples were reported after having been followed-up in an automatized blood culture system (BACTEC 9240, Becton Dickinson Diagnostic Instrument Systems, USA). All blood culture samples were evaluated as positive, bacterial growth having been evalauted with Gram staining and subculture passage. All the blood culture samples which were evaluated as negative at the end of the incubation period had been evaluated with Gram staining and passage cultures for false negative. Standard methods were used in accordance with the recommendations of the guidelines in culture studies performed for the other system samples. Bacterial pathogens were identified according to standard conventional microbiological methods (Winn et al., 2006).

RESULTS

Of the cases, 89 were from the pediatrics and the pediatric emergency departments and five were from other clinics. Thirty four of the cases were female and 60 of them were male. Most of the cases (67%) were aged 0 to 12 month. A single sample was sent from 26 cases, and 2 or more samples were sent from 68 cases for bacteriological examination. Of the samples, 60 were blood, 58 were CSF, 48 were urine, 36 were tracheal aspirate and six were other clinical materials. Bacterial growth was reported in 21 (35%) out of 60 blood cultures, in 6 (10.3%) out of 58 CSF samples, in 9 (18.8%) out of 48 urine samples, in 29 (80.6%) out of 36 tracheal aspirates and in 1 (16.7%) out of 6 the other samples. Microorganism growth was reported as follows: a single type of microorganism growth in 19 out of 21 positive blood cultures and multiple growth in 2; a single type of microorganism growth in four out of six positive CSF cultures and multiple growth in 2; a single type of microorganism growth in 22 out of 29 positive tracheal aspirates and multiple growth in 7, and a single type of microorganism growth in all of the 9 positive urine cultures. While *Candida species* and *Pseudomonas aeruginosa* were isolated most in the tracheal aspirate samples, coagulase negative staphylococcus (CNS) and *Klebsiella pneumoniae* were isolated from the blood cultures. Distribution of single and multiple microorganisms isolated from postmortem samples have been demonstrated in Tables 1 and 2.

Seventy-eight out of a total of 94 cases were found to have the demand for culture in at least one sample in antemortem period. In the antemortem period while bacterial growth was reported in 10 (14.9%) out of 67 blood samples, in 12 (21.1%) out of 57 urine samples, in 23 (65.7%) out of 35 tracheal aspirate samples, and in 3 (27.3%) out of remaining 11 other samples, growth was reported in none of the 10 CSF samples. Furthermore, it was seen that the clinical samples which had been cultured in the antemortem period and the ones cultured in the postmortem period were not always the same and there was more than one culture demand from the same sample at different time intervals in the antemortem period in some cases.

DISCUSSION

Postmortem bacteriology is valuable as it provides information about whether death has resulted from infection or not in forensic cases and about the presence of nosocomial microorganisms and the microorganisms originating from the surroundings of the hospital (Tsokos and Püschel, 2001; Roberts, 1998). In this study, the results of postmortem culture samples sent from different clinics were evaluated. The samples which were sent following autopsy as forensic cases were excluded. The vast majority of the samples were sent from the department of pediatrics. It was determined that the blood and the CSF samples were the most commonly sent samples in this study and the tracheal aspirate and blood samples were found to be in the foreground among the samples in which bacterial growth was detected. In the literature, there are studies investigating the bacteriological examinations, particularly in sudden infant death syndrome or unexpected infant death cases (Weber, 2010; Prtak et al., 2010; Pryce et al., 2011; Highet et al., 2009).

Assessment of the results of the growth in postmortem cultures comprises various difficulties. Interpretation of culture results has been reported to be a main problem in postmortem microbiological assessment (Caplan and Koontz, 2001). Many environmental factors also play a role besides the importance of the detailed data about the case, the type and the time of obtaining samples in the interpretation of the results. Preserving the integrity of the samples obtained for microbiological examination in postmortem and forensic cases and doing this under certain conditions are of great importance. In particular,

Table 1. Type and number of single isolated microorganisms from postmortem cultures.

Microorganism	Blood	CSF	Urine	Tracheal aspirate	The other specimens*
Gram negative					
K. pneumoniae	5	1	3	1	-
E. coli	2	-	2	1	-
E. aerogenes	1	-	-	1	-
S. marcescens	-	-	-	1	-
P. aeruginosa	3	-	-	4	-
A. baumannii	-	1	-	1	-
A. lwoffii	-	-	-	2	-
S. maltophilia	-	-	-	2	-
Gram positive					
CNS	5	1	-	1	-
S. aureus	-	-	-	2	-
E. faecium	1	-	1	-	-
Fungus					
C. albicans	1	-	1	4	
Candida spp (non *C. albicans*)	1	1	2	2	1
Total	19	4	9	22	1

*The other specimen: Pleural fluid, peritoneal dialysis fluid, peritoneal aspirate, intubation canula swab.

Table 2. Distrubiton of polymicrobial isolated microorganisms from postmortem cultures.

Microorganism	Blood	CSF	Urine	Tracheal aspirate	The other specimens*
Upper resp. normal flora** + *Candida spp.*	-	-	-	2	-
E. coli + *K. pneumoniae*	-	1	-		-
A. lwoffii + *Candida spp.*	-		-	1	-
P. aeruginosa + *K. pneumoniae*	-	1	-	-	-
K. pneumoniae + *Candida spp.*	-	-	-	1	-
E. aerogenes + *Candida spp.*	1	-	-		-
P. aeruginosa + *K. Pneumoniae* + *E. coli*	-	-	-	1	-
P. aeruginosa + *S. aureus*	1	-	-	-	-
Upper resp. normal flora**	-	-	-	2	-
Total	2	2	-	7	-

*The other specimen: Pleural fluid, peritoneal dialysis fluid, peritoneal aspirate, intubation canula swab. **Upper resp. normal flora: members of the upper respiratory tract normal flora

the elapsed time, temperature, humidity, and contamination are important and may affect the results and the assessment (Mazuchowski and Patricia, 2005; Caplan and Koontz, 2001). Although the correct time for obtaining the samples is controversial, this condition is one of the important factors for interpretation of the results. Postmortem spread from intact intestinal walls has been shown in different studies. There are publications available recommending the transport of the samples for bacteriological examination within the first postmortem 24 h besides those reporting that obtaining blood samples within the first postmortem 15 or 48 h would reduce the false positivity rate due to postmortem bacterial invasion (Tsokos and Püschel, 2001; Lobmaier et al., 2009; Saegeman et al., 2009). In our study, we could not obtain data about the time when the samples were obtained. However, it was considered that the samples had been obtained just after death as the vast majority of the cases were children and hosopitalized patients. Hence, positive growth was considered to be related to true positivity or

contaminations occuring during obtaining the samples rather than postmortem bacterial invasion.

It is recommended to obtain postmortem blood and spleen samples concurrently for the diagnosis of bacteremia. It has been reported that the results would be significant if the same microorganism grows in all of the blood samples obtained from different sites or both the blood and the spleen samples (Roberts, 1998; Mazuchowski and Patricia, 2005). While a single sample was sent in 26 cases, two or more clinical samples were sent for bacteriological examination in 68 cases. In our study, it was seen that no spleen samples of the cases had been sent and that the blood samples were obtained from a single site. In this study, the most commonly isolated two microorganisms were found to be CNS and *K. pneumoniae* in 21 blood cultures. In one study, the blood culture was reported to have been obtained from 65 cadaver tissue donors within the postmortem 24 h and after 24 h and CNS was reported to have been isolated most in both groups (Saegeman et al., 2009). In another study, it was reported that the bacterial pathogen was isolated in 216 (54.5%) out of 396 culture samples obtained from the heart blood following autopsy. In the same study, non-fermentative Gram negative rods were reported to be the most commonly isolated microorganisms from the blood cultures followed by *K. pneumoniae* (Srifeungfung et al., 2005). In a study conducted with SUDI cases, the blood culture was reported to be evaluated in 105 out of 116 cases, CSF in 79, and ear smears in 31 cases, and the potential pathogen microorganisms were reported to have been isolated in 16% of blood cultures, in 6% of CSF cultures and in 48% of ear smears (Prtak et al., 2010).

The lower respiratory tract and the lungs are sterile under normal conditions. However, a few bacteria may be present in the bronchial secretions at the time of death and growth related to these bacteria has been reported after death (Morris et al., 2007). In previous studies, microorganisms located in the mouth were shown to be detected in the lungs after death. Positive culture results can be obtained in the lungs without pneumonia findings due to the drainage of oral secretions (Roberts, 1998). Obtaining samples from the upper respiratory tract as soon as death occurs before being transferred to the morgue is important as it reflects the flora at the time of death. It has been reported that nasopharyngeal *S. aureus* carriage is 50% in the first 3 months of life and decreases thereafter to be around 30% between 3-12 months. *S.aureus* carriage has been blamed for the etiology of sudden infant death syndrome (Morris et al., 2007). In the study of Goldwater (2009), he reported that *S.aureus* was isolated at a rate of 10.7% in sterile sites in SIDS cases, 18.7% in infection-related SUDI cases and that *S. aureus* was not isolated in sudden accidental death. In the same study, it was reported that *S. aureus* was detected in the lung samples at a rate of 34.4% in infection-related SUDI cases and at a rate of 21.5% in SIDS cases, and there was no difference between the two groups in terms of isolation. It was emphasized that *S. aureus* should be kept in mind in the etiology of SUDI and that it would be beneficial to demand microbiology consultation and to assess the microbiological findings before the last report is written in the autopsies performed for SUDI cases (Goldwater, 2009). In another systematic retrospective study, autopsy was performed in 507 SUDI cases between 1996 and 2005, samples were obtained for bacteriological examination and the results of 470 cases were evaluated. In the same study, *S. aureus* was reported to be isolated most in blood cultures, CSF and spleen samples, and mainly in the lung samples (Weber et al., 2008). The vast majority of the cases had come from the department of pediatrics in our study; however, it is not known whether the cases were diagnosed with SUDI or not. Tracheal aspirate samples were the samples in which postmortem growth was detected most in our study. While *Candida* spp. and *P. aeruginosa* were isolated most in these samples, *S.aureus* was isolated in two samples. Some of the subjects were found to have been hospitalized for a long time before death. Similar microorganisms were seen to have been isolated also in the antemortem tracheal aspirates of the subjects in whom these microorganisms had been detected. Colonization and infection should be discriminated well in patients with prolonged hospitalization. It should be kept in mind that the isolated microorganisms may be the causative agent and may also be colonized.

In a retrospective study, the antemortem blood culture results within the previous 10 days of 111 adults who had undergone autopsy and the postmortem blood culture results were compared and false positivity was found as 11.7% in the antemortem cultures and 13.5% in the postmortem cultures. *S. epidermidis* was determined to be the microorganism which grew most and polymicrobial growth was observed, although infrequent (Hove and Pencil, 1998). In our study, the data obtained from the results of cultures sent during the hospital stay before death in 94 cases were analyzed. However, it was found that the culture demand of some cases were not present in the antemortem period, and that the sample types before and after death were not consistent (example while blood and tracheal aspirate cultures had been demanded before death, only CSF culture was demanded after death, etc.) or more than one culture demands were present at different intervals in the antemortem period of the hospitalized patients. This situation was limitation of our study. Due to all these reasons, it was considered that comparing the antemortem and postmortem growths including the whole group would not be proper. Assessment of the antemortem and postmortem growths at a case level may provide an opinion, but consistency of both culture results is not sufficient to decide about the cause of death, although significant. Presence of antemortem infection

findings, whether the microbial agent isolated before and after death is a member of the flora or not, and the presence of colonization should be discriminated. It is important to investigate the evidence of infection and inflammation in the postmortem histopathological examinations and take the antemortem clinical findings and history into consideration while interpreting the microbiological results of the samples obtained just after death or during autopsy. Consistency of postmortem culture results with clinical, radiological, laboratory and histopathological results renders a more realistic assessment. Bacterial positivity in postmortem culture samples may be related with true positivity, agonal spread, postmortem translocation or contamination (Roberts, 1998; Morris et al., 2006, 2007). Therefore, the postmortem environmental conditions and the time should be considered to play a role in bacterial invasion when evaluating the postmortem samples sent for examination. It should be kept in mind that determination of the cause of death based only on the postmortem culture results may be misleading and other postmortem examinations and antemortem history are also important.

In conclusion, the culture results of the postmortem samples sent to our laboratory were evalauted retrospectively and the pathogenic microorganisms which may be clinically important were isolated from various samples. However, it was considered that the usability of these culture results as evidence of infection as the cause of death could be controversial. It was concluded that the postmortem bacteriological examinations and the culture results would be significant when evaluating the antemortem diagnosis, time and conditions of obtaining the samples, time to reach the laboratory and other histopathological findings together.

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