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Evaluation of specific marker CK13 and CK10/13 combined with APM staining for the diagnosis of amniotic fluid embolism and aspiration

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ABSTRACT

Objective: To explore the value of CK13 (Rab) and CK10/13(Mab) as objective and specific biomarkers combined with Alcian-Phloxine-Martius yellow (APM) staining for the diagnosis of amniotic fluid embolism (AFE) and amniotic fluid aspiration (AFA).
Methods: A retrospective study of forensic autopsy cases of 148 neonatal deaths and 19 maternal deaths in the perinatal stage was conducted at the Institute of Pathology and Forensic Medicine, Zhejiang University. Medical records were reviewed and monoclonal antibody for CK13 (Rab) and CK10/13 (Mab) as immunostaining of amniotic fluid squamous cells, APM staining, and Hematoxylin and Eosin (HE) staining were used to diagnose the AFE and AFA. Descriptive statistics of the patient population were analyzed using SPSS 20.0 software.
Results: Immunoreactivity of CK13 and CK10/13 specifically identified squamous cells of all the AFE and AFA cases. The amniotic fluid squamous cells were stained positive in a deep brown color with the monoclonal antibody to CK 13 and CK10/13 whereas the endothelial cells and alveolar epithelial cells were negative. There was no CK13 or CK10/13 expression in the non-AFE and non-AFA cases. With APM staining keratinized squamous cells were pink and mucus was blue-green in marked contrast with the surrounding tissue, which improved the detection rates of both keratinized squamous cells and mucus.
Conclusions: CK13 (Rab) and CK10/13 (Mab) are valuable and reliable biomarkers of amniotic fluid squamous cells. APM reveals enriched mucus and keratinized squamous cells of amniotic fluid. Immunohistochemical detection of CK13 and CK10/13 combined with APM staining can improve the accuracy and reduce the difficulty in the diagnosis of AFE and AFA.

1. Introduction

Amniotic fluid embolism (AFE) is a rare but major obstetric catastrophe that occurs in 1/8000 to 1/80,000 deliveries [1–3] and accounts for 10–26% of maternal deaths in Western countries [4,5]. In China, although mortality rate caused by AFE has declined from 20.7% in 1996 to 15.0% in 2010, it is still a major cause of maternal deaths [6]. The first case of amniotic fluid entering the maternal circulation was described by Meyer in 1926 [7]. The AFE and its pathogenicity was not widely recognized until 1941 when an autopsy series of 8 women who died of sudden shock during labor reported fetal debris in the maternal pulmonary circulation [8]. Clinical diagnosis is always difficult and is essentially one of exclusion based on clinical presentation.

Similarly, amniotic fluid aspiration (AFA) is an infrequent but life-threatening disease affecting some term infants born with neonatal intrauterine distress. AFA can cause many harmful complications, such as neonatal respiratory disorders characterized by asphyxia, anoxia and acidosis, and a serious amniotic fluid aspiration syndrome (meconium aspiration syndrome, MAS). AFA is present in 8–20% of all deliveries [9,10], especially in term and

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post-term infants, while the mortality rate reaches 39.3% in MAS and 13.5% in pure AFA [11].

The diagnosis of AFE and AFA has traditionally been made at autopsy when fetal materials such as squamous cells, mucin, and meconium are identified by HE stain in the maternal pulmonary circulation (AFE), or in the neonatal airways and alveolar space (AFA) [12]. Identification of fetal materials such as squamous cells usually depends on subjective analysis and experience of pathologists. The lack of an objective laboratory "gold standard" for the diagnosis of AFE and AFA not only hampers early clinical recognition of the disease [13], but also hinders correct diagnosis at autopsy. Sometimes, as a result of lack of effective and objective testimony in AFE and AFA cases, obstetricians and pediatricians are caught in medical malpractice dispute with legal litigation because such a case of AFE or AFA often results in sudden unexpected death of the mother or the infant. Medico-legal investigation of such death to determine AFE and AFA as cause of death has long been a challenge to forensic pathologists. It is not uncommon that AFE and AFA is unrecognized or misdiagnosed at autopsy even by experienced pathologists, especially when the tissues and organs have autolyzed because of delayed autopsy.

In a previous study [14], we used both conventional murine monoclonal antibodies (mainly Pan-CK, LMW-CK, HMW-CK, and CK10/13) and a new rabbit monoclonal antibodies (mainly CK4-CK8, CK10, and CK13-CK20) to screen for a specific marker of amniotic fluid cells and found that CK10/13 (MAb, clone ID DEK-13) and CK13 (RAb, clone ID EPR3671) are specific for squamous cells extracted from maternal amniotic fluid. They are strongly expressed in squamous cells and negative in endothelium and alveolar epithelium. In the present study, we further explored the value of CK13 and CK10/13 as specific markers in the diagnosis of AFE and AFA using immunohistochemical investigation. In addition, we carried out a retrospective review of cases to identify the risk factors for fatal AFE and AFA.

2. Materials and methods

Formalin-fixed paraffin-embedded lung tissue specimens were collected from forensic autopsy cases of 148 neonatal deaths during 1991 to 2011 and 19 maternal deaths occurred intrapartum or postpartum during 1989 to 2008 at Institute of Forensic Medicine, Zhejiang University. The postmortem interval at time of autopsy was from 0.5 h to 7 days.

Alcian blue, Phloxine, and Martius yellow (APM) were from Aladdin (Shanghai, China). The APM reagent was made up as described previously [15]. Sections were de-waxed to water and placed in 3% acetic acid buffer for 1 min. Then Alcian-blue acetic acid staining was performed for 20 min, Phloxine for 5 min, and Martius yellow for 15 s. Hematoxylin was used to stain nuclei.

A mouse monoclonal anti-CK10/13 antibody (working liquid, Zhongshan, Beijing, China) and a rabbit monoclonal anti-CK13 antibody (1:250 dilution from initial liquid, Epitomics, Hangzhou, China) were used for the immunohistochemistry. The tissues were fixed in 10% formaldehyde, embedded in paraffin, and cut into 4-μm sections. The sections were incubated overnight at 4 °C with the antibody. Detection was performed with a polymer detection kit (Elivision™ Super, Maixin, Fuzhou, China) according to the manufacturer's instructions, followed by reaction with DAB and counterstaining with hematoxylin. Normal lung tissues from other autopsy cases were used as a negative control.

Routine HE staining, APM staining, and immunostaining of CK13 (Rab) or CK10/13 (Mab) were done on all the 148 neonatal cases and the 19 maternal cases. The diagnosis of AFE and AFA were established by two experienced pathologists based on the stains and clinical history.

Medical history of the all neonatal deaths and maternal deaths were reviewed. In the maternal death group, maternal age, gestational age (GA), mode of delivery, survival time, and presence of disseminated intravascular coagulation or major hemorrhage were included in the analysis, while in the neonatal death cases, birth weight, GA, gender, presence of congenital anomalies were documented.

All statistical analyses were performed using SPSS 20.0 software (SPSS, Chicago, IL, USA) to describe the patient population. Values are reported as percentages and means, and were compared by the independent-samples t-test, Fisher's exact test, or the χ^2 test, as appropriate. A P value <0.05 was considered to be statistically significant. This study was exempt from approval by the local Ethics Committee of Zhejiang.

3. Results

Routine HE staining, APM staining, and immunostaining of CK13 (Rab) or CK10/13 (Mab) were done on all the 148 neonatal cases and the 19 maternal cases. The diagnosis of AFE and AFA were established by two experienced pathologists based on the stains and clinical history. Among 148 neonates, 80 neonates (54.1%) were diagnosed AFA, and the cause of death in the non-AFA group included congenital heart disease, neonatal sepsis, fetal distress, and birth defects. Among 19 mothers, 8 (42%) were diagnosed AFE and the other 11 mainly died of hemorrhagic shock and puerperal sepsis.

The HE histological examination of lung samples revealed the presence of fetal squamous cells and debris in the pulmonary vessels (AFE group, Fig. 1a); and in the airways and alveolar space (AFA group, Fig. 2a). While with APM staining, squamous cells and mucus were much more clearly evident in comparison with HE (Figs. 1b and 2b). Fetal squamous cells were stained pink, mucus was blue-green, and microthrombi were yellow with APM staining. Among the 8 AFE patients, mucus strands were found in 5 cases, fetal squamous cells in all 8 cases, and microthrombi in 3 cases with APM staining. Since the main component of fetal aspiration is mucus, the use of APM staining in the diagnosis of amniotic fluid aspiration is much more reliable than HE stain.

In the more difficult cases of AFE and AFA, immunostaining of CK13 (Rab) or CK10/13 (Mab), however, clearly identified fetal squamous cells with brown cytoplasmic granules in the squamous cells. CK13 (Rab) and CK10/13 (Mab) cytokeratin stains showed intense intravascular positivity in AFE group (Fig. 1c and d). In the AFA cases, immunostaining of CK13 (Rab) or CK10/13 (Mab) was strongly positive in the bronchial and alveolar spaces (Fig. 2c and d). Immunohistochemical staining techniques have greatly improved the accuracy in the diagnosis of AFE and AFA.

The clinical characteristics of the neonates were presented in Table 1. Of the 80 neonates who died from AFA, the mean birth weight was 3.17 ± 0.60 kg ($P < 0.05$) and gestational age was 39.80 ± 2.22 weeks ($P < 0.05$) when compared with non-AFA group whose mean birth weight was 2.81 ± 0.75 kg and gestational age was 37.69 ± 2.97 weeks. The mean birth weights of different gestational age groups (GA < 37; $>37 < 42$; >42) were as follows: 2.35 ± 0.49 kg; 3.18 ± 0.57 kg; and 3.65 ± 0.13 kg for AFA neonates and 2.31 ± 0.19 kg; 2.95 ± 0.70 kg; and 3.35 ± 0.21 kg for non-AFA neonates, respectively. AFA neonates with gestational age more than 42 weeks have highest mean birth weight. Among the AFA neonates, 49 cases had a GA >37 weeks (between 37 and 42 weeks) and 3 cases >42 weeks, indicating that AFA was more likely to occur in term and post-term infants ($P < 0.05$).

The clinical characteristics of the mothers were presented in Table 2. Of the 19 mothers, 10 were nine months pregnant and 3 were eight months pregnant at the time of delivery; data for the other 6 cases was not available. The mean survival interval