

Immunohistochemical study of thyroid transcription factor-1 and surfactant-associated protein A for investigation of peripheral airway structure in perinatal fatality

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Immunohistochemical study of thyroid transcription factor-1 and surfactant-associated protein A
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(5) Abbreviations

TTF-1, anti- thyroid transcription factor-1; PE-10, anti- surfactant-associated protein A

Abstract

We studied histopathological findings of the lungs in four cases where there was a suspicion of infanticide whereby an autopsy was performed. All four babies were born in full term delivery. It was difficult to discern whether the peripheral airways of the lungs were open or closed with conventional histological examination. Therefore immunohistochemistry with a combination of anti- thyroid transcription factor-1 (TTF-1) and anti- surfactant-associated protein A (PE-10) was used in order to elucidate the fundamental structures of the peripheral airways. TTF-1 highlighted nuclei of Clara cells and type II alveolar cells. The findings of immunohistochemistry with TTF-1 enabled us to more objectively recognize the peripheral airways: respiratory bronchioles and alveolar ducts, even in collapsed lungs. PE-10 was expressed in the cytoplasm of Clara cells, type II alveolar cells, and the substance of air space. Aspect for immunohistochemistry with PE-10 appeared to be granular in closed air space, whereas it appeared to be stretched membranously along the interalveolar septa in open air space. These findings suggest that application of immunohistochemistry with TTF-1 and PE-10 is a useful diagnostic tool in judging perinatal fatality.

Keywords

Perinatal fatality; Peripheral airway structure; Immunohistochemistry; Anti- thyroid transcription factor-1; Anti- surfactant-associated protein A

1. Introduction

Diagnosing whether a new born infant is stillbirth or a live birth is one of the challenges in forensic pathology. Since both macroscopic and microscopic findings of the lungs are not necessarily definitive, we face the trouble of making a precise judgment in cases of suspected infanticide.

In making a distinction between stillbirth and live birth, forensic pathologists have directed their attention to the following findings: shape, color, and consistency of the lung; hydrostatic test [1]; localized expansion of respiratory bronchioles and alveolar ducts; inflammation of the plica vocalis; and inflammation of the outer umbilical ring [2]. Among these, expansion of the peripheral airways including respiratory bronchioles and alveolar ducts has been regarded as the most important. However, it is sometimes difficult to recognize the fundamental structures of the peripheral airways with conventional histological stainings, especially in collapsed lungs.

In view of these considerations, we have undertaken the present study to evaluate the immunohistochemical expression and distribution of TTF-1 and PE-10; in order to determine whether immunohistochemical findings with these antibodies are useful for recognizing the fundamental architecture of the peripheral airways in four cases where there was a suspicion of infanticide whereby an autopsy was performed. Here, we report that immunohistochemistry with a combination

of TTF-1 and PE-10 could give us useful information about the status of the peripheral airway structure.

2. Materials and methods

2.1. Autopsy cases

The studied judicial autopsy cases involved four newborn infants where there was a suspicion of infanticide. This study was undertaken at the Department of Legal Medicine of the University of Toyama between 1995 and 2007 (Table 1). Because of illegitimate pregnancy, the mothers delivered each baby by themselves without medical assistance.

2.2 Immunohistochemistry

The sample tissues of the lung were fixed with the buffered 10 % formalin and embedded in paraffin and were available in each case. The tissue blocks were sectioned at a thickness of 4 µm for the immunohistochemical study.

The tissue sections were immunostained by the peroxidase method (EnVision System; DAKO,

Carpinteria, CA) to evaluate the localization and degree of reactivity for a mouse monoclonal antibody against thyroid transcription factor-1 (TTF-1, DAKO Inc., Carpinteria, CA; dilution, 1 : 100) [3], and a mouse monoclonal antibody against surfactant-associated protein A (PE-10, DAKO Inc., Kyoto, Japan; dilution, 1 : 100) [4]. In addition, a mouse monoclonal antibody against cytokeratins (AE1/AE3, DAKO Inc, Kyoto, Japan; dilution, 1 : 200) was used to detect foreign bodies in the aspirated amniotic fluid. Briefly, after deparaffinization the sections were treated for antigen retrieval with a tris buffer solution (TRS) in a wet chamber under a microwave three times for 5 min each [5]. The sections were allowed to cool at room temperature for 30 min. The sections were washed in TRS and sequentially treated with 3 % H₂O₂ for 15 min to exhaust endogenous peroxidase. After washing with TRS and blocking of nonspecific binding of the secondary antibody with 5 % normal horse serum for 30 min, the sections were incubated with a primary antibody in a wet chamber under a microwave for 10 min. After three washes with TRS, the peroxidase-labeled polymer from the EnVision System kit was applied for 10 min in a wet chamber under a microwave. After washing in TRS, the color was developed with the Vector VIP substrate kit (Vector Laboratories Inc., Burlingame, CA), and the sections were counterstained with Mayer's hematoxylin.

3. Results

3.1. Case summary

Forensic pathological findings together with Hydrostatic test and immunohistochemistry in each case are listed in Table 1. Histopathological findings in each case depended on examination of the sample tissues from each of the lung lobes, and were found to be similar.

3.2. Histopathological findings

The lumens of bronchioles were wrinkled and the surrounding alveolar spaces were collapsed in Case 1, which made it difficult to understand the structure of peripheral airways (Fig. 1. A). In contrast, air spaces were open enough to recognize the structure of the peripheral airway in Case 2 (Fig. 1. D). The extent of openness in the peripheral airways showed regional differences in Case 3 (Fig. 2. G). In this case, putrefaction slightly progressed. Geographical heterogeneity of openness in the peripheral airways appeared to be greatly strengthened by mechanical ventilation after delivery in Case 4 (Fig. 2. J).

A small amount of aspirated amniotic fluid was observed in the bronchioles and alveolar spaces in Case 1 and 3 (Fig. 1. A and 2. G). Massive amniotic fluid with admixture of squamous epithelia was observed in Case 4 (Fig. 2. J).

3.3. Immunohistochemical findings

Immunohistochemical stainings with TTF-1 and PE-10 were shown in Fig. 1. B, 1. E, 2. H, 2. K, and 1. C, 1. F, 2. I, 2. L, respectively.

Type II alveolar cell and Clara cell revealed a distinct reactivity for TTF-1 in their nuclei. However, the surface epithelial cells of bronchioles were negative for TTF-1. Therefore, those positive for TTF-1 enabled us to recognize respiratory bronchioles and alveolar ducts, even in collapsed lung tissue (Fig. 1. B, 2. H, and 2. K).

Alveolar substance floating in alveolar spaces, which was related to surfactant-associated protein A, showed a positive reaction for PE-10 in all cases. As shown in Fig. 1. C, 2. I, and 2. L the substances positive for PE-10 in closed alveolar spaces appeared to be granular, whereas those in open alveolar spaces appeared to be membranously stretched along the interalveolar septa (Fig. 1. F).

In Case 4 with massive aspiration of amniotic fluid, aspirated squamous epithelia in the peripheral air spaces were highlighted by immunostaining with AE1/AE3.

4. Discussion

The present study demonstrates that immunohistochemistry with TTF-1 and PE-10 enables us to more easily understand the fundamental structure of peripheral airways in forensic cases where there

is a suspicion of infanticide.

TTF-1 is selectively expressed in the thyroid, lung and diencephalons [6] and has been shown to be important in the activation of pulmonary-specific differentiation genes [7]. Determining whether the peripheral airways are open or closed has been problematic, especially in cases where there is a discrepancy between the result of the Hydrostatic test and histological findings, such as Case 4. In the present study, immunohistochemistry with TTF-1 clearly showed the location of respiratory bronchioles and alveolar ducts with the arrangement of Clara cells and type II alveolar cells in line.

In forensic pathology, Zhu and his colleagues have already applied surfactant immunohistochemistry with PE-10 to a large series of routine cases including perinatal death, asphyxia, CO intoxication, respiratory distress and so on [8, 9, 10]. Chronological development of surfactant apoproteins (SPs) in the lung has been demonstrated by several researchers [8, 11]. mRNA of SPs is detected in the 18-20th week, and SPs are immunohistochemically demonstrated after the 31st week with a peak increase occurring in the 35-36th week of gestation. All of the infants in the present study were born in full term delivery without developmental retardation. Those aspects for immunohistochemistry with PE-10 appeared to be granular in closed air spaces, whereas they appeared to be stretched membranously along the interalveolar septa in open air space. Therefore, we conclude that PE-10 could be a useful tool for structural comprehension of the peripheral airways.

In conclusion, we required more objective evidence demonstrating the status of the peripheral airways after conventional histological examination. By conventional histological examination and

this immunohistochemical study, we identified that the babies, except for that of Case 2, were stillborn, and that the baby in Case 2 was a live birth, although she might have been born in asphyxia. Immunohistochemistry with a combination of TTF-1 and PE-10 is useful for the comprehension of peripheral airway structure and is a useful diagnostic tool for judging perinatal fatality.

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Figure legend

Fig. 1.

Hematoxylin-eosin stain (A) and immunohistochemical staining with TTF-1 (B) and PE-10 (C) in Case 1. The peripheral airway structures including alveolar space, alveolar duct, and respiratory bronchiole are closed. Clara cells and type II alveolar cells positive for TTF-1 in nuclei are arranged in a line, which enables us to recognize the respiratory bronchioles and alveolar ducts. Substances with positive reaction for PE-10 are aggregated in closed alveolar spaces and appear to be granular.

Hematoxylin-eosin stain (D) and immunohistochemical staining with TTF-1 (E) and PE-10 (F) in Case 2. The peripheral airways are open. Clara cells and type II alveolar cells positive for TTF-1 are arranged in a line. Substances with positive reaction for PE-10 are stretched membranously along the interalveolar septa in open air spaces.

Bars indicate 100 μ m.

Fig. 2.

Hematoxylin-eosin stain (G) and immunohistochemical staining with TTF-1 (H) and PE-10 (I) in Case 3. The peripheral airways are not completely open. Clara cells and type II alveolar cells positive for TTF-1 are arranged in a line. Substances with positive reaction for PE-10 show a granular pattern. Putrefaction slightly progresses. Nevertheless, reactivity for TTF-1 and PE-10 is preserved.

Hematoxylin-eosin stain (J) and immunohistochemical staining with TTF-1 (K) and PE-10 (L) in

Case 4. The peripheral airways are open in part because of mechanical ventilation. Bronchiole and neighboring alveolar spaces are filled with foreign bodies composed of squamous epithelia due to aspiration of amniotic fluid. Clara cells and type II alveolar cells positive for TTF-1 are arranged in a line. Substances with positive reaction for PE-10 show a granular pattern.

Bars indicate 100µm.

Table 1
Case summary

Case	Gender, Height, Body weight, Time following delivery	Cause of infant death*, Abnormality of delivery	Color of lungs, Hydrostatic test	Status of peripheral airway on HE	<u>Immunohistochemistry</u>	
					TTF-1	PE-10
1	Male, 49 cm, 3,140 g, Half a day	Circulatory insufficiency, Transverse lie	Reddish, Negative	RB and AD, closed; AMF, (+)	Positive	Granular pattern
2	Female, 52 cm, 2,810 g, 1 day	Circulatory insufficiency, Premature separation of the normally implanted placenta	Slightly reddish, Positive	RB and AD, open; AMF, (-)	Positive	Stretched pattern
3	Male, 48 cm, 3,110 g, 3 days	Circulatory insufficiency, (-)	Reddish, Negative	RB and AD, incompletely open; AMF, (+)	Positive	Granular pattern
4	Female, 47.5 cm, 2,415 g, Half a day	Massive aspiration of amniotic fluid, Looping of the umbilical cord	Slightly reddish, Positive	RB and AD, open and closed**; AMF, (++)	Positive	Granular pattern

* Every baby was free from either malformation or fatal diseases.

** Mechanical ventilation was used on infant for half an hour after delivery.

Abbreviations: RB, respiratory bronchiole; AD, alveolar duct; AMF, detection of foreign bodies probably deriving from aspiration of amniotic fluid.

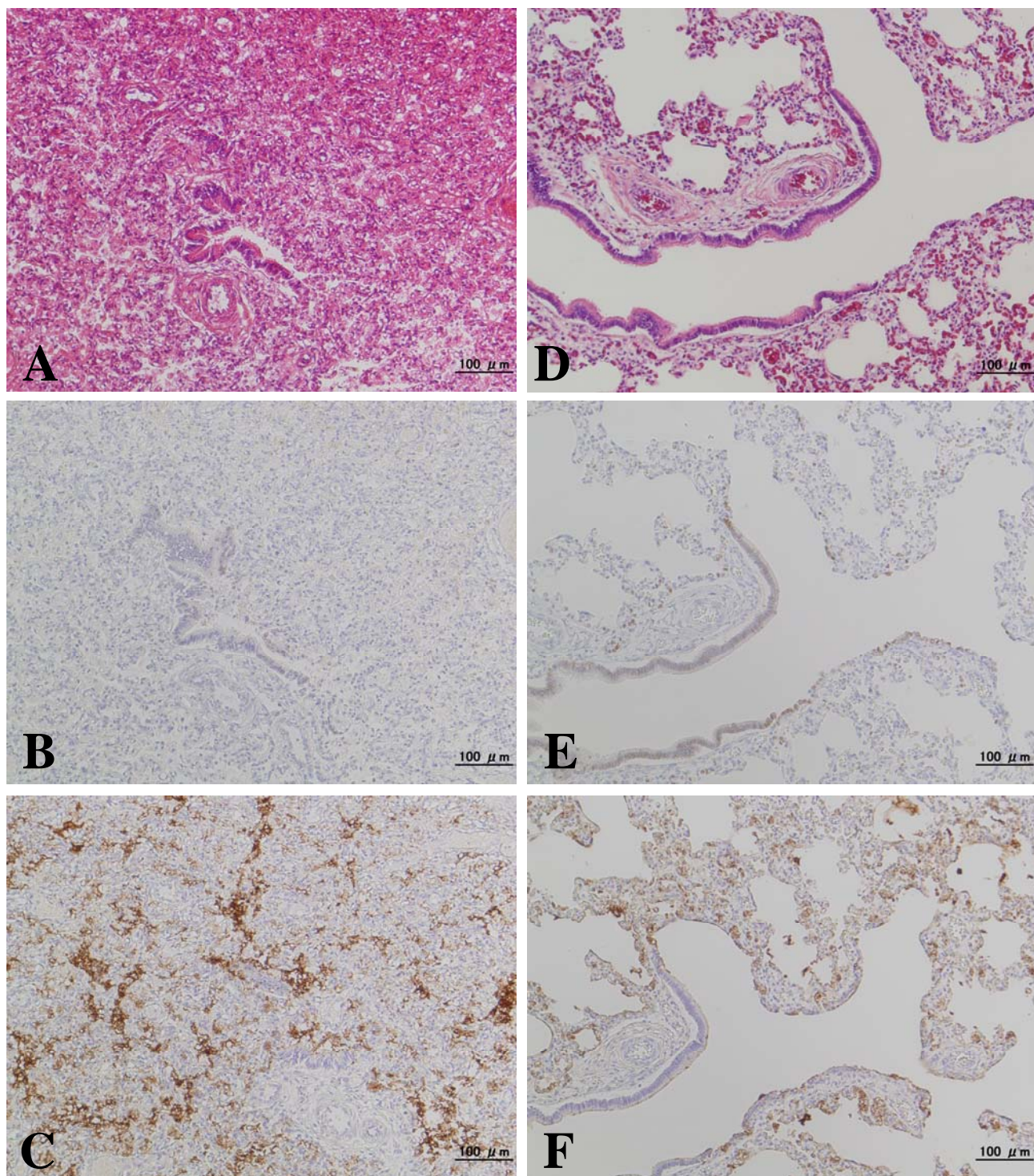


Fig. 1.

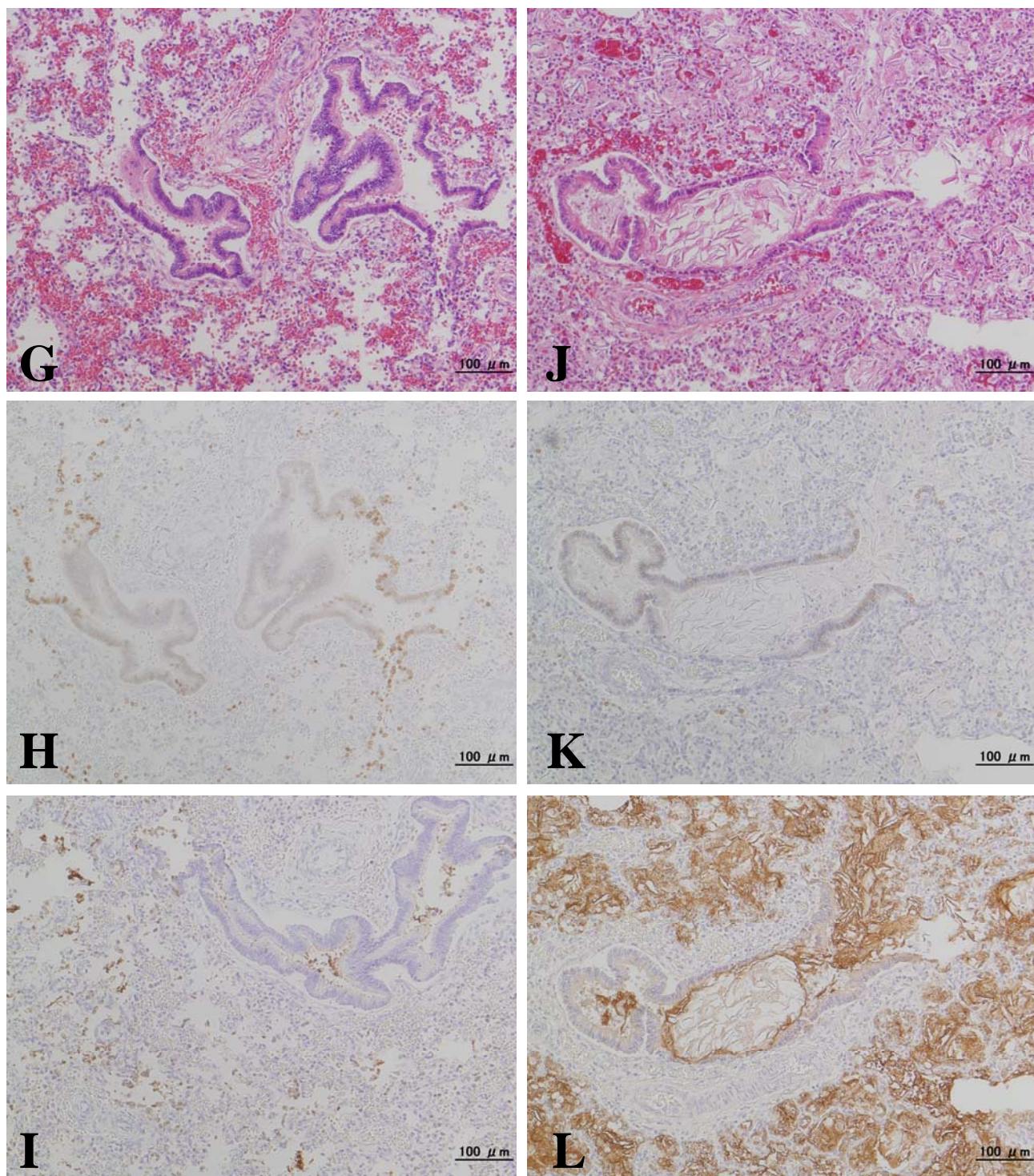


Fig. 2.