Asian Archives of Pathology 2012; Vol. 8 No.2, 77-83

Case report

Pulmonary alveolar proteinosis coexisting with homozygous hemoglobin E

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ABSTRACT

Background: Pulmonary alveolar proteinosis (PAP) is an uncommon lung disorder of obscure and possibly diverse causes characterized by abnormal accumulation of lipoproteinaceous surfactant material in alveoli. The prevalence of acquired PAP has been estimated to be 0.37 per 100,000 populations. We present a case of PAP coexisting in homozygous hemoglobin (Hb) E.

Case presentation: A 13-year-old girl who had been diagnosed as homozygous Hb E. She presented with dyspnea and cough. Thoracotomy was performed due to abnormal mediastinal mass disclosed in CT scan. However, only slightly thymus gland enlargement was found then wedge lung biopsy was performed. Examination of the sections of lung revealed alveoli diffusely filled with eosinophilic granular material. Bronchoalveolar lavage was also performed. Cytopathologic evaluation revealed a milky appearance with microscopically amorphous granular material with positive periodic acid-Schiff stain and scant chronic inflammatory cells. Findings were consistent with PAP. The clinical condition of the patient was stable after treatment.

Keywords: pulmonary alveolar proteinosis, bronchoalveolar lavage, cytology, hemoglobin E

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INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare disorder characterized by the accumulation of surfactant proteins and phospholipids within the alveolar spaces. PAP can be classified into congenital, secondary and acquired forms (1). Acquired PAP is divided into two forms on the basis of clinical features: idiopathic PAP and secondary PAP. Idiopathic acquired PAP was originally defined in 1958 (2) and it comprises more than 90 % of cases (1). Secondary PAP is related to a number of clinical situations, including pharmacologic immunosuppression, dust exposure or toxic fumes, infections, and hematologic malignancy (1-4). The prevalence of idiopathic PAP has been estimated to be 0.37 per 100,000 persons (1). The median age at the time of diagnosis is 39 years (1). The series reported in the literature suggest a male predominance (male:

female ratio 3:1) that may be linked to tobacco smoking (1, 5). Several recent studies in transgenic mice and human have demonstrated that impairment of surfactant clearance by alveolar macrophages as a result of inhibition of the action of granulocyte-macrophage colony-stimulating factor (GM-CSF) by blocking autoantibidies may underlie many acquired idiopathic PAP. Secondary PAP may result from functional impairment of GM-CSF receptor on alveolar macrophages and/or an abnormal signal transduction pathway after interaction of GM-CSF and its receptor that is associated with hematologic disorders (1, 3-4).

A diagnosis of PAP is based on the examination of bronchoalveolar lavage (BAL) specimen and/or an examination of lung biopsy. Cytologic findings of BAL specimens from patients with PAP have been reported (6-7) but most reports come from western countries and reports in Asian populations are rare (4, 6-12).

Hemoglobin (Hb) E is very common in parts of Southeast Asia and expresses varied mutation. It results in a heterogenous group of disorders whose phenotype range from asymptomatic to severe. Hb E trait and homozygous Hb E are mild disorders (13-14). Although secondary PAP associated with hematologic disorders such as chronic granulocytic leukemia with progressive myelofibrosis, myeloproliferative disorders and myelodysplastic syndrome (3-4, 9, 12) has been reported, but coexistence of acquired PAP with hemoglobinopathy, in particular homozygous Hb E, has never been reported to the best of our knowledge.

In this paper, we report a case of homozygous Hb E coexisting with acquired PAP which was diagnosed by histological findings of lung biopsy and subsequently whole lung lavage for treatment.

Case report

A thirteen-year-old girl, known case of Hb E (Hb baseline 13.5 g/dL), was referred to our hospital in 2009 from a local hospital in Chonburi province. She presented with dyspnea and cough for one month. The provisional diagnosis from that local hospital was severe pneumonia with a suspected of pulmonary tuberculosis. Then antituberculosis and antibiotics treatment had been given for two months but her clinical condition was getting worse. The CT scan of chest found anterior mediastinal mass with extensive increased opacification with air-bronchogram and bronchiologram in both lungs. Therefore, thoracotomy was performed and revealed slightly thymus gland enlargement, then wedge lung biopsy was concurrently performed. Examination of sections obtained from left upper lobe revealed alveoli diffusely filled with eosinophilic granular material admixed with rare cholesterol cleft, however, no evidence of inflammation or vasculitis was found.

She was referred to our hospital for further management. Physical examination on the first admission, pale conjunctiva but no clubbing of fingers was noted. Bilateral fine crepitation was audible at both chest walls. Spleen was also palpable 1 cm. below left costal margin. No other abnormality was detected. Ultrasonography of abdomen revealed splenomegaly without other focal mass. Laboratory examinations showed decreased in hemoglobin levels but other serologic study showed negative antiHIV, negative for mycoplasma antibody, and negative blood PCR study for CMV. CMV viral load was less than 600 copies per milliliter of blood plasma. Ig A, Ig G, and Ig M level were within normal range. Blood antinuclear antibody (ANA) test showed positive at 1:80 dilution with fine speckled pattern. C3 and C4 level were within normal range and negative p-antineutrophil cytoplasmic antibody (ANCA) and c-ANCA. High-resolution CT study

revealed decreased air-space with consolidation patchy geographic area of ground-glass opacity.

BAL with whole lung lavage was performed for cytologic study and treatment, respectively, and it also showed negative for ANA test. BAL specimen was opaque milky in appearance and revealed a large amount of amorphous granular cyanophilic and basophilic extracellular material with few alveolar macrophages and lymphocytes (figure 1). This material showed brightly granular stained for periodic acid-schiff (PAS) reaction, with or without diastase digestion (figure 2). Cytologic findings were compatible with pulmonary alveolar proteinosis. Initial serum lactate dehydrogenase (LDH) level was 776 U/L and the level was consequentially decreased after BAL treatment to 549U/L on second week of admission, 409 U/L on third week of admission and 175 U/L before discharge from hospital. In addition, obtained sections of lung biopsy specimen were reviewed and the histologic findings revealed structurally intact alveoli diffusely filled with eosinophilic granular material (figure 3 and 4). This material was also positive for PAS stain. No cholesterol cleft was found. Neither viral-cytopathic effect nor fungal infection was found in PAS and Grocott methenamine silver (GMS) stains. The ultrastractural study by transmission electron microscopy (TEM) showed lamellar bodies (Figure 5) Findings were compatible with alveolar proteinosis. However, further investigation for surfactant protein A (SP-A) or granulocyte macrophage colony-stimulating factor (GM-CSF) analysis measurement of GM-CSF level in BAL fluid or blood could not be performed because unavailable laboratory for these tests in Thailand. According to the proper clinical setting and results of investigations, the patient was diagnosed as acquired pulmonary alveolar proteinosis.

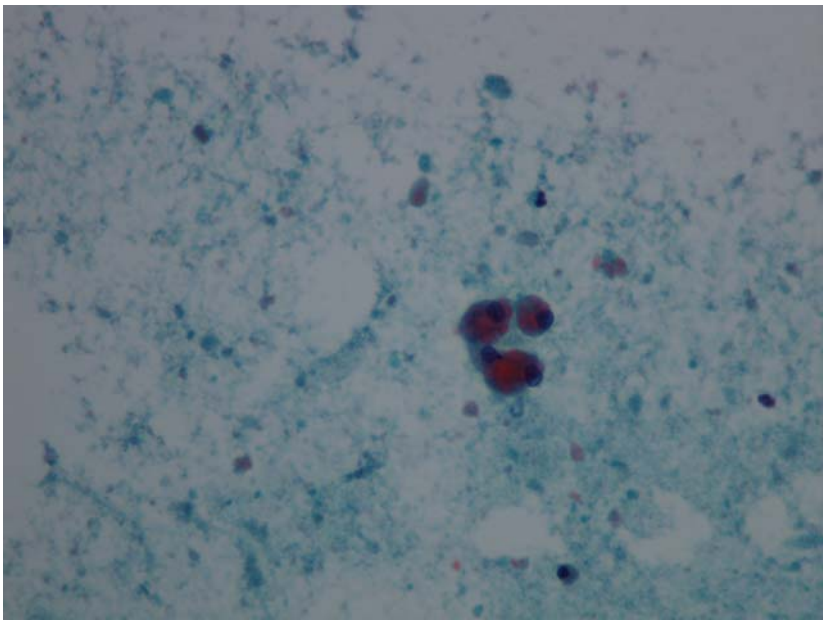

Figure 1 Papanicolaou-stained smear of bronchoalveolar lavage fluid shows mainly amorphous granular and globular extracellular material with few foamy alveolar macrophages (original magnification x400).

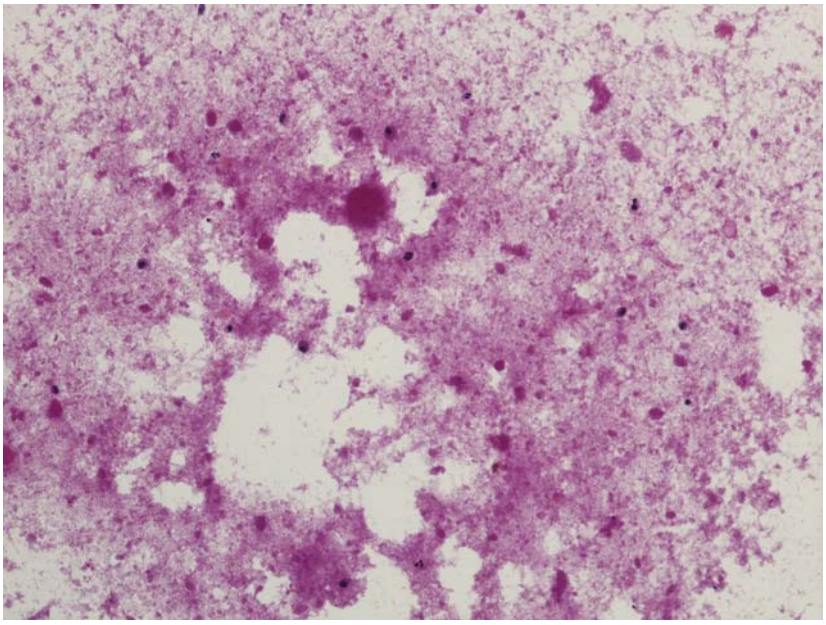

Figure 2 The globular material shows strong PAS stain (original magnification x100).

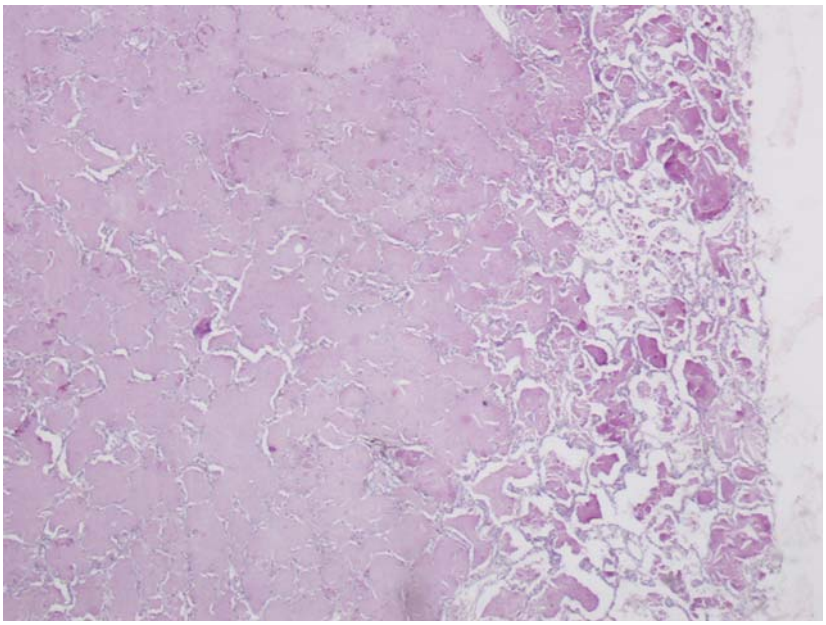

Figure 3 Section of the open lung biopsy reveals alveoli diffusely filled with abundant granular eosinophilic material with remarkable large lamellar concretions within the alveoli near pleural surface (Hematoxylin-eosin-stained, original magnification x40).

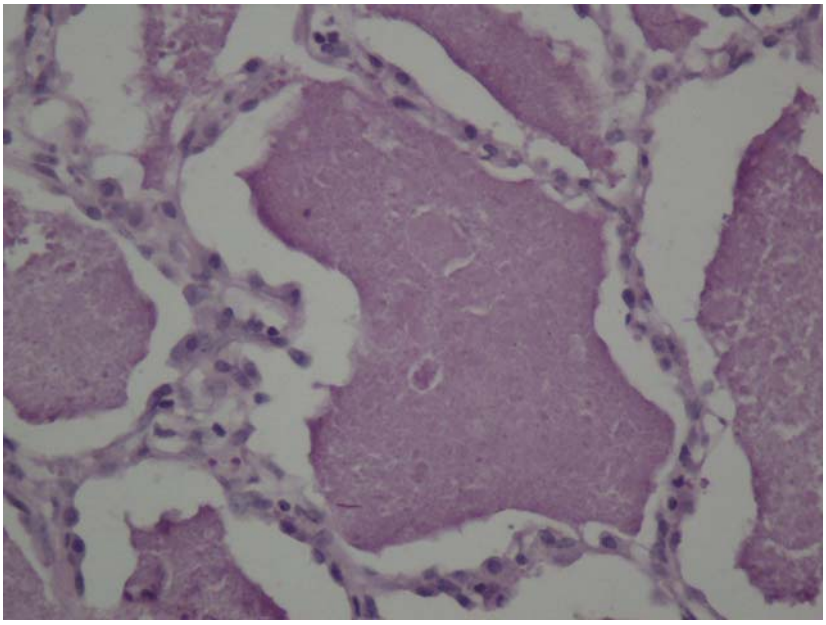

Figure 4 ection of the open lung biopsy reveals alveoli diffusely filled with abundant granular eosinophilic material with scant alveolar macrophages (original magnification x400).

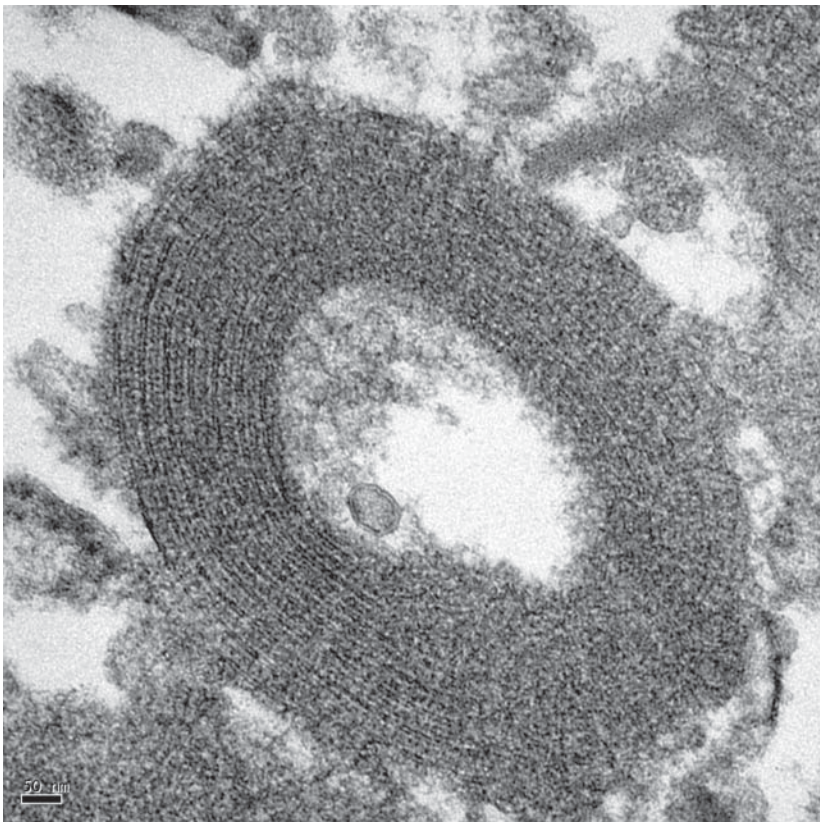

Figure 5 The ultrastructural study of BAL demonstares lamellar body which is characteristic of alveolar proteinosis. (TEM X 40,000)

DISCUSSION

PAP is an uncommon lung disease and is included in the differential diagnosis of patients all ages presenting with dyspnea. PAP was first described in 1958 by Rosen and colleagues (2) as a rare lung disorder in which the alveoli are filled with PAS-positive proteinaceous material, rich in lipid. The exact etiology is not clear and probably is multifactorial (1-8). Initially, it was thought that an inhaled irritant (e.g., silica) or infectious agent induced an excessive production of the natural material lining the alveoli caused PAP (10). However, the inability to find such agents in the lung-biopsy specimens of most PAP patients failed to support this idea (1). Recent observations in animal experiments and human suggest that the acquired PAP caused by abnormal surfactant homeostasis due to presence of the neutralizing anti-GM-CSF autoantibodies (1, 5). These autoantibodies against GM-CSF cause defects in the functioning of alveolar macrophages, including impairment of the catabolism of surfactant lipids and proteins and disruption of surfactant homeostasis (1, 5).

Although in the past a diagnosis was made by open lung biopsy, but in recent year BAL cytology and/or transbronchial biopsy have been useful for the diagnosis. Electron microscopy (EM) demonstrating lamellar bodies can provide ultrastructure confirmation (7, 11). However, this expensive and time-consuming procedure is not available to many hospitals and is unnecessary in most cases. Recently, BAL with lower cost and convenience has become the standard for both the diagnosis and treatment for PAP (6, 7, 11). On cytologic examination of BAL specimen based on Papanicolaou's stain reveals amorphous, globular, cyanophilic or basophilic PAS-positive diastase resistant material, generally, with low numbers of inflammatory cells and macrophages. This globular material of BAL specimen can be of significant value in making a diagnosis of PAP, especially when the number of globules is more than 18 (6).

In differential diagnosis, some of the conditions to be considered include pulmonary edema, pneumocystis pneumonia, amyloidosis, amiodarone toxicity, and other pulmonary infections such as nocardiosis, histoplasmosis, cryptococcosis, aspergillosis, mycobacterial diseases, candidiasis, and multiple viral infections (6-7, 11). Pulmonary edema lacks the globular material and cellular debris seen in PAP. Pneumocystis pneumonia can be distinguished from PAP by a more dramatic clinical presentation, exudates that appear finely vacuolated or foamy, and significantly more inflammatory background. With carefully search, the exudates are composed of one or two tiny dot-like trophozoites, measuring 0.5 to 1 μm in diameter, which could be confirmed by GMS stain. Amyloidosis has also been rarely reported in BAL specimen and amyloid usually has a "harder", more discrete appearance than fluffy amorphous material of PAP (7). The Congo red stain produces a characteristic reddish color with yellowish-green birefringence for amyloid. Amiodarone toxicity is other rare potential causes of false-positive clinical and cytologic diagnoses of PAP (7, 11). The clinical manifestations of pulmonary toxicity include progressive dyspnea and cough. BAL specimen reveals many large mono and multinucleated macrophages with finely foamy cytoplasm. Osmophilic lamellar inclusion bodies have also been observed in lysosomes of alveolar macrophages by EM, corresponding to foamy cytoplasm in macrophages by light microscopy that are similar to the ultrastructural appearance of PAP (5).

In conclusion, we report a case of homozygous Hb E coexisting with acquired PAP that is rarely found in Thailand. The coexisting between PAP and hemoglobinopathy in this case is still

unexplainable and await for further investigation.

ACKNOWLEDGEMENT

We thank Dr. Woranart Ratanakorn, Dr. Ratana Tantranon, and other doctors in Chonburi hospital who performed tissue sampling and analysis and took responsibility for patient management. We also thank the cytotechnicians and laboratory workers in our department for skillful technical support.

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Asian Archives of Pathology 2012; Vol. 8 No.2, 84-87

Case report

Sudden Unexpected Death With Marked Decomposition: A Case Report Of Intracerebral Hemorrhage By Contracoup Injury

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ABSTRACT

The author reported a deceased died in her room. The only loud crying had been heard by her neighboring resident, two days before her body was found in decomposed condition. The postmotem inquest was performed by a police officer and a forensic physician. Her body was transferred to Siriraj Hospital for full autopsy. The autopsy revealed a large part of scalp contusion at left parieto-occipital area without skull fracture. Intracerebral hemorrhage of right frontoparietotemporal lobe was found associated with "contracoup lesion".

Key words: intracranial hemorrhage, contracoup lesion, autopsy, postmortem inquest, criminal procedural code

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INTRODUCTION

When unnatural death is found in Thailand, the “post-mortem inquest” must be done according to Section 148 of the Criminal Procedure Code[1]. The unnatural death can be recognized when the dead bodies are found in serious injuries which were caused by various types of weapons , traffic accidents, etc and it is quite obvious that the cause of deaths are unnatural[2]. For the decomposed cases, it is very difficult for the physicians to find out the causes of death. However, the forensic physicians who have had experience in postmortem inquest and specific focus in full autopsy could find the evidences and causes of death. The author reports a specific decomposed case caused by intracranial contracoup hemorrhage.

Case Report: (F-550916)

History:

The deceased was a 43- year- old female employee living in an apartment. A man in the neighboring room said that she came to her room in the evening of July 4, 2012. He heard a loud meaningless crying around 2.00 am. of July 5, 2012 and thereafter nobody could see her again.

Two days later (July 7, 2012) around 7 o'clock, owners of the apartment detected bad smelling from her room and tissue debris oozing from the front door. After breaking the door, the deceased body with marked decomposition was found lying on the floor. The local police and forensic physician were called at 7.30 am., postmortem inquest was performed at 11.50 am. and found no

definite cause of death. Her body was then transferred to Siriraj Hospital for complete autopsy. The external examination revealed that the cadaver was that of about 40 year- old female, 155 cm. long, with black hair and 10-15 cm. long. There were only short pyjamas (short trousers) on her body.

The cadaver was markedly decomposed. The expected time of death was about more than 48 hours before the deceased found. The external examination showed rotten scalp, detached hair from scalp due to decomposition. dark color of skin of the body, trunk and limbs. The nail of fingers and toes painted pinky-gold color without cracking. No serious injury on the body and limbs was found. Arms and legs were stretched out without deformity. No odor or smelling of specifific chemicals was detected from the deceased body except marked decomposition. There was no serious injury on the head . Marked contusion was located at left parieto-occipital area, measured approximately 10X7 cm. No skull fracture was found.The dark purple-black stain was found at dura mater of right fronto-parieto-temporal area, estimated about 10 cm. in diameter directly opposite to the site of scalp contusion at left parieto-occipital area. The brain looked like "sticky liquid", grey color without specific pathology in both hemispheres, except at right fronto-parieto-temporal area revealing dark or grey-brown color. Intracerebral hemorrhage as estimatedly about 10-15 cc. of decomposed blood. in right fronto-parieto-temportal lobe was found. Intraventricular hemorrhage could not be identified. Vertebrae and ribs were normal. Marked decomposition of both lungs was detected, with dark liquid fluid in pleural cavity. The heart was found decomposed but preserved structure. No significant pathology of coronary vessels was found. Other organ in thorax revealed unremarkable, except for decomposition. Liver, spleen, kidneys, intestines and other organs in the abdomen were decomposed. The uterus could be identified in normal size. No evidence of pregnant uterus was found. Other organs in abdominal and pelvic cavities were found decomposed. The laboratory investigations were performed by sending the decomposed liver, kidney and muscle (thigh) for analysis in toxicological laboratory. The results revealed that no drugs or toxic substances were found. The cause of death was concluded as Intracerebral hemorrhage of right fronto-parieto-temporal lobe, right hemisphere.

DISCUSSION

When the physician finds the dead and decayed or decomposed body in post-mortem inquest, he should consider that the body was dead by “unnatural death” until prove otherwise. The cadaver must be transferred to perform full and standard autopsy in specific and authorized place by law, such as governmental hospital (in accordance with Section 151 and 152 of the Code of Criminal Procedure) [1] and law of *Thai Medical Council*[3]. After the full autopsy, many cases may not find any pathology to be the causes of deaths due to marked decomposition of the cadavers. However, if there are some organs remained, the forensic pathologist and forensic physician may be able to find out the evidences which are the causes of death. The following items should be put in consideration for the decomposed cadaver.

First consideration: The cadaver revealed markedly decomposed without any findings of:

a. violent wound and injury,

b. angulation of limbs and neck,

c. tearing of clothing for suspected of sexual case.

As the above finding, natural causes of death such as acute cardiac failure may be possible but the suicde or homicide could not be excluded.

Second consideration: Is(Are) there any evidence(s) of internal violent wound or injury by toxic substance(s) (without bad smelling)?

Third consideration: In Thailand, there has been a large group of death found as sudden unexpected death, brugada (sudden unexpected death syndrome or SUDS), which has been no obvious pathology from complete autopsy[4].

So, by the above reasons of decayed or decomposed body, the cadaver must be transferred to the authorized place for full autopsy.

The autopsy of this case was performed under Section 151 and 152 of the Code of Criminal Procedure. From autopsy and pathological examination we could postulate the cause of death as head injury of left side (contracoup lesion) which caused by the right side intracarnial hemorrhage due to falling down on the ground with strongly violent impact on the right side of her head. The force was transmitted to the opposite side of the brain as contracoup lesion. This pathology was not as severe as the assault, hit by a wooden stick or iron bar at the head. The question was raised as to whether this hypothesis was true or not. This issue may be explained by a few reasons:-1). Most common contracoup lesion of the brain is epidural hemorrhage, more than subdural hemorrhage. However, there have been some reports presented the contracoup could cause bleeding in the brain (intracerebral or intraventricular hemorrhage) as well. 2). The “Lucid Interval” could be considered after the head injury. 3) Intracerebral hemorrhage caused the lesion at left side of scalp. This meant that there had been a spontaneous intracerebral hemorrhage at right hemisphere which caused the cadaver to fall down with her head knocked to the ground. The pathology of this assumption has been conflicted because of her age (only 43 years old) and her healthy life history (no any chronic disease: such as stroke or cardiac disease).

In Thai Law, Criminal Procedural Code[1], the cause and manner of death in this case are very important for the police as well as the insurance company. The insurance company would pay the compensation depending on the manner of death and condition(s) in report of forensic physician as followings:-

a) If the left side head injury caused right side intracranial hemorrhage, the insurance company would pay as the rate of accident.

b) If right intracerebral hemorrhage caused the left side contusion, the insurance company would pay as only natural death rate which is markedly different from the accident rate.

Therefore, the conclusion of the cause and manner of death in the decomposed body should be crucial, and full complete autopsy should be performed.

SUMMARY

If we find the cadaver in markedly decomposed condition, we must assume that the cadaver died as an unnatural death. The postmortem inquest should be performed . Most of the cases would not be able to find the causes of death at the scene. So, the cadaver must be transferred to official hospital or specific institution for performing complete autuosy. After full autopsy, we may find that the causes and manner of most cases couldn’t be obtained. However, some cases may leave evidences as in our presenting case to be able to find possible cause of death.

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