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Surfactant Protein Type-A in Diagnosis of Drowning Cases

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Abstract

Background Drowning is a main universal community health problem. In medico-legal practice, the autopsy diagnosis of drowning presents one of the major problems especially when there is delay in recovering the victim from water.

Objective To gather the autopsy findings with the serology test "Surfactant-associated protein A" (SP-A) procedures to reach more accurate diagnosis of drowning and to clarify the significance of serology test (SP-A) procedures.

Methods This study was performed at Medico-legal Directorate (MLD) in Baghdad for (12) months within the period from 1/1/2018 to 31/12/2018. Full proper autopsy including external and internal examination of the body for all cases was performed, after obtaining complete medico-legal history, in addition to serology test (SP-A) procedures to determine the cause of the death as due to drowning.

Results The study included (60) cases, (52) males and (8) females with their ages ranged between (15-44 years old) for male, while ages ranged between (1-44 years old) for females. Drowning was the cause of death in all cases. The most important result of this study is that the serum SP-A concentration showed increment alongside with period that had been passed since the event of drowning. The highest value was (1042.167 ng /L) after 48 hours from the event.

Conclusion The concentration of SP-A increases with increasing duration of immersion in water and an important marker in the diagnosis of drowning together with the autopsy findings.

Keywords Medico-legal study, SP-A, drowning

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List of abbreviations: ALI = Acute lung injury, ARDS = Acute respiratory distress syndrome, MLD = Medico-legal Directorate, SP-A = Surfactant-associated protein A

Introduction

Drowning is a main universal community health problem. It is the process of experiencing respiratory impairment from submersion / immersion in liquid as stated by world health organization [1,2]. In a medico-legal view, drowning is a type of asphyxia due to aspiration of fluid either water, milk, oil ...etc instead of air and immersion of whole body or nose and / or mouth under the level of fluid [3-6]. In drowning there is relation between liquid and air junction at the entrance of the airway prevents breathing air [7]. Drowning could be considered as a mixture of mechanical presence of water within the respiratory system (mechanical asphyxia) with liquid and electrolyte changes depending on the medium (either sea or fresh water) in which immersion has occurred [8]. Drowning cases are usually accidental in nature, they could be suicidal and rarely homicidal [6]. Drowning is a common method of suicide in India and (35%) of cases in Austria are positive

for alcohol [7]. It is the second leading cause of accidental death in children at 14 years old [9]. In medico-legal aspect, the problems with drowning start with the scenes involving long sections of sea, rivers or lakeshore, mainly in cases underwater disaster identification [10,11] and failure to find a body quickly [12].

Autopsy signs include immersion and drowning signs. Immersion signs are maceration (corrugation) of the skin, which is the first sign that starts within minutes in warm water, while in cold water it would be visible after a variable time, the minimum is (4 or 5) hours depending upon temperature other study suggest a longer duration from (12 to 48) hours. Maceration is obvious in hands and soles, the skin becomes wrinkled, pale and wet so-called "washerwoman's skin". Maceration could be seen also on the extensor surface of knees and elbows [9] and it was appeared in summer days within (30-60) minutes, in mild atmosphere in (3-4) hours and is delayed in cold season [13].

Cutis anserine (goose-flesh) is a common sign in immersed bodies [9].

It is usual for most corpses to float or hang in water with buttocks uppermost, while the head and limbs are hanging down. The hypostasis in cadaver pulled out cold water is pink color [2,9].

Mud, coal-slurry, oil, silt or sand present on the body, in addition to other artefacts such as seaweed, waterweed, algae. Mud may be adherent to the whole-body surface and clothing [9].

The corpses are colder in the depths of rivers, seas, canals, and ponds than the outer atmosphere. Contraction of scrotum may happen before or after death [8].

Algae growth on the skin is helpful to determine the position of cadaver. They may be found in trachea and stomach as a sign of immersion, but not of active inhalation of fluid [13-16].

In medico-legal practice, the autopsy diagnosis of drowning is one of the major problems specifically when there is delay in recovering the victim [17,18]. Drowning signs include froth in the air passages as a positive sign in fresh bodies. Froth is edematous fluid from the lungs and consists of a proteinaceous exudate and surfactant mixed with the water of the drowning medium [3,9]. It is white in color and may be pink or red-tinged, due to slight mixing up with blood from intrapulmonary bleeding. [9,13].

Generally, the weights of a lung in drowning is about 600-700 g, whilst the non-drowned is about 370-540 g [19].

Froth also observed in epilepsy, electrical shock, drug intoxication and cardiogenic pulmonary edema [8,19]. The lungs of a drowning victims commonly look like those seen in deaths associated with severe pulmonary edema, as in cases of arteriosclerotic heart disease [17,20].

The most important internal organ to observed and the most information about the cause of death in drowning are lungs. They are distended brick red in color, with signs of emphysema [9,13,21]. The edematous fluid in the bronchi locks the passive collapse that normally occurs at death, holding the lungs in the inspiratory position [9,15]. This is a positive sign of drowning at autopsy [15].

The increase in weight of lungs and is due to asphyxia and aspiration of water [20].

The heart and great veins are dilated and engorged with fluid blood, especially the right side, but this is non-specific [13,21,22]. Pleural fluid accumulation is associated with drowning, the volume of which controversially being said to reflect the post-mortem interval [8]. Subpleural hemorrhages (Paultauf's spots) may reflect hemolysis within intra-alveolar spaces and have been described in (50-60%) of cases of drownings [8,22].

In stomach, there is Wydlers sign due to swallowing of water or Mallory-Weiss syndrome (esophageal mucosal tear) [23].

Miscellaneous signs in drowning include bloody or watery fluid in the intracranial sinuses, engorgement of solid organs, reduced weight of the spleen, Tardieu spot on organ and muscular hemorrhages in the neck and back

and all are additional physical signs of drowning [8].

Cadaveric spasm is a positive sign and may be see in one or both hands. There may be grass, herbs, or gravel in the fist of the victim [8,9,15,20].

Surfactant-associated protein A (SP-A) is a lipoprotein & an innate immune system collectin. It is water-soluble. It is part of the innate immune system [21]. Pulmonary surfactant is covering the surface of the alveoli and essential for normal lung function as it maintains alveolar stability and prevents alveolar collapse by reducing surface tension at the air-liquid interface [22,23]. SP-A are very important constituents of pulmonary surfactant [24,25]. It is a hydrophilic and large collagen-like glycoprotein produced by the alveolar type (II) cells and bronchiolar epithelial Clara cells [26]. Function of SP-A in the alveolus is to facilitate the surface tension-lowering properties of surfactant phospholipids, to regulate surfactant phospholipid synthesis, secretion, recycling by alveolar type (II) pneumocytes and alveolar macrophages and to resist the inhibitory effects of plasma proteins released during lung injury [27]. SP-A is dependent on the presence calcium because it plays a strong role in the structure and function of pulmonary surfactant after secretion into the alveolar space [28].

Surfactant loss or changes of the proteinaceous fluid into the air spaces lead severe pathological significances and SP-A altered in a variety of pulmonary diseases such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). [29]. Clinical ALI and the ARDS represent a common response of the lung to a variety of insults, including trauma, infection, aspiration of water or gastric contents, inhalation of toxic gases, and pneumonia [30]. SP-A concentration changes in bronchoalveolar lavage fluid (BAL) and in serum have been investigated in ARDS/ALI found useful since it is correlated significantly with the level of SP-A [31]. In Japan, SP-A immunohistochemically studied as a marker of pulmonary function and possible usefulness for postmortem investigation of death involving asphyxiation and respiratory distress [32]. Increase membranous or linear SP-A staining on the intra-alveolar surface indicated as an increase of surfactant secretion due to various fatal stresses for example acute myocardial infarction and carbon monoxide (CO) intoxication [33,34]. SP-A has been also observed in a different immunohistochemical distribution pattern such as aggregated granular staining distributed both intracellular and extracellular in the intra-alveolar space, but the exact mechanism of massive aggregates production remains to be determined [35]. This aggregated form of SP-A useful tool to distinguish mechanical asphyxia from other hypoxic cases and the effect of drugs and poisons on respiratory function [36]. Massive aggregates of granular SP-A staining were found in (70.4%) of mechanical asphyxia cases e.g. hanging, strangulation, smothering and choking. A high score of intra-alveolar aggregates of SP-A was more frequently observed also in freshwater (66.6%) than saltwater (6.25%) drowning victims. (34) Not all cadavers recovered from water or found near water are drowned [37].

Drowning remains one of the most difficult diagnoses in forensic pathology because macroscopic and microscopic autopsy findings are unspecific [38]. In medico-legal aspect, histology and immunohistochemistry are significant tools for study of pulmonary tissue. An ideal diagnostic marker for drowning still needs to be developed, other conditions that lead to increase SP-A are asphyxiation, drowning and respiratory distress syndrome [39].

The aim of this study is to clarify the significance of serology test of SP -A to strengthen the diagnostic of fatal drowning cases.

Methods

Site and duration

Medico-legal Directorate (MLD) of Baghdad for one-year duration from 1\1\2018 till 31\12\2018.

Subjects
Sixty victims of drowning.

Inclusion criteria
Cases with circumstantial evidence favoring drowning death.

Exclusion criteria
Decomposition.

Methods
External examination, which include examination of clothes and external signs, followed by internal examination and taking blood samples for serology test of SP-A (Eliza test), and this includes:

1. Ten ml of blood were taken from the heart.
2. Samples were left for 10-20 minutes to be clotted.
3. Centrifugation at 300-400 RPM for 5-10 minutes.
4. Serum samples were taken in test tube and stored in deep freezing at -20 °C
5. Samples were sent to the serology lab for SP-A investigation.

SP-A investigation
ELISA Kit done by dilution standard solutions: In this kit provides one standard original concentration. Users may independently dilute in small tubes following the chart below:

- 480 ng/L Standard No.5 120 µl Original Standard + 120 µl Standard diluents
- 240 ng/L Standard No.4 120 µl Standard No.5 + 120 µl Standard diluents
- 120 ng/L Standard No.3 120 µl Standard No.4 + 120 µl Standard diluent
- 60 ng/L Standard No.2 120 µl Standard No.3 + 120 µl Standard diluent
- 30 ng/L Standard No.1 120 µl Standard No.2 + 120 µl Standard diluent
- Standard solution No.5 No.4 No.3 No.2 No.1

The number of stripes needed is determined by that of samples to be tested added by the standards. It is recommended that each standard solution and each blank well be arranged with multiple wells as much as possible.

Sample injection

1. Blank well: Do not added sample, anti SP-A antibody labeled with biotin and streptavidin-HRP; added chromogen reagent A & B and stop solution, each other step operation is the same.
2. Standard solution well: Added 50 µl standard and streptomycin-HRP 50 µl (biotin antibodies had united in advance in the standard, so no biotin antibodies are added).
3. Sample well: Added 40 µl sample and then 10 µl SP-A antibodies, 50 µl streptavidin-HRP. Then covered it with seal plate membrane. Shake gently to mix. Incubate at 37 ℃ for 60 minutes.
4. Distilled water for later use.
5. Washing: carefully removed the seal plate membrane, drained liquid and shacked off the remainder. Fill each well with washing solution, let stand for 30 seconds, then drained. Repeat this procedure five times then blot the plate.
6. Color development: First added 50 µl chromogen reagent A to each well, and then added 50 µl chromogen reagent B to each well. Shaked gently to mix. Incubate for 10 minutes at 37 ℃ away from light for color development.
7. Stop: Added 50 µl Stop Solution to each well to stop the reaction (color changes from blue to yellow immediately at that moment).
8. Assay: Taken blank well as zero, measure the absorbance (OD) of each well under 450 nm wavelength, which should be conducted within 10 minutes after had added stop solution.

According to standards concentrations and corresponding OD values, calculated the linear regression equation of the standard curve. Then according to the OD value of samples,

calculated the concentration of the corresponding sample.

Results

Throughout the total period of collecting samples for study, which extended from 1st January to 31st of December of 2018, death cases due to drowning was recorded in only (134 out of 6591) cases referred to the MLD in Baghdad and the ages of victims drowning were among (4-45 years) and history were taken from family about diseases and appeared most of cases not complain from chronic diseases of lungs while control cases were road traffic accident. Drowning was the 10th cause of death being responsible for only (2%) of cases referred to MLD during the period of study. This study was showed that the SP-A concentration increment with Increasing duration of immersion in water as the concentration at the beginning of the first 30 minutes was (10.655 ng/l) and at the end of 30 minutes (36.95 ng/l) while the concentration of SP- A was within 48 hours stay in water at the beginning (303.938 ng/l) and the end of it, (1402.167 ng/l) and all drowning cases were not decomposed because the decomposing was affected on serum SP-A result and SP-A was test for drowning diagnosis not definitive test as in Table (1) and Figure (1).

Table 1. Concertation of SP-A in serum with times in drowning cases

	Time					
	30 m	**60 m**	**360 m**	**12h**	**24h**	**48 h**
SP-A (ng/l)	10.655	53.406	101.737	160.708	179.966	303.938
	11.459	53.707	103.335	165.381	180.424	319.041
	13.088	62.175	105.414	165.463	186.932	333.573
	22.139	63.905	112.225	172.842	224.153	587.953
	31.575	69.231	117.936	175.398	237.214	673.967
	35.199	70.04	118.955	177.42	246.638	858.834
	36.395	70.544	121.064		249.434	1402.167
		70.571	122.112			
		70.688	125.651			
		74.692	126.922			
		76.115	127.082			
		77.114	130.15			
		77.844	130.635			
		82.351	135.483			
		85.246	144.092			
		88.97	146.544			
			152.21			

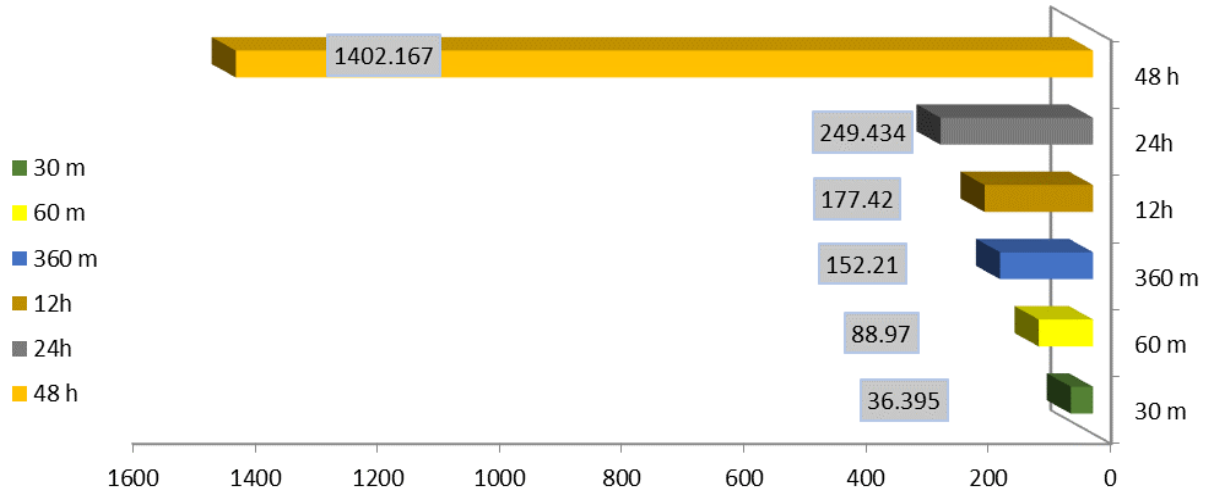

Figure 1. Highest value Concertation of SP-A (ng/l) in serum with times in drowning cases

Discussion

The SP-A concentration increment with longer period of time increasing duration of immersion in water before recovering the body from water, we observe a concentration at the beginning of 30 minutes was (10.655 ng/l) concentration while at the end of 30 minutes (36.395 ng/l) and also the concentration at the beginning of 48 hours (303.938 ng/l), at end was (1402.167 ng/l), the reason is inhaling water during drowning leads to the rupture the wall of alveoli, thus leading to the exit of SP-A into the blood stream while staying in water with longer period of time lead to increase damage to the wall of the alveoli and concentration of SP-A in the blood was increment. Some victims were having lung diseases that exerted an effect on alveolar wall leading to increment the concentration of SP-A in the serum. This study agreed with study in Italy the SP-A as a marker of asphyxiation and drowning. The postmortem diagnosis of drowning continues to be one of the most difficult in forensic pathology because of unspecific autopsy findings This study shows that the concentration of SP-A increment with prolongation of immersion in it is a good strengthening the diagnosis of drowning cases as it is a good marker of alveolar injury [39].

This study disagreed with study in Italy; the massive and dense intra-alveolar SP-A aggregates can only support the final diagnosis of drowning as well as duration and severity of respiratory distress. Intense membranous or linear SP-A pattern as well as low granular scores can only support the detection of pulmonary edema fluid on the intra-alveolar surface [40].

As a conclusion, this study showed an increment of the SP-A concentration with stay victims for long time inside water and serum SP-A test for diagnosis of drowning cases but not fully specific but is a good marker of alveolar injury.

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Author contribution

Dr. Hussein collected cases and wrote the research, Dr. Hashim and Dr. Abdulla did the statistic to research.

Conflict of interest

None to be declared.

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