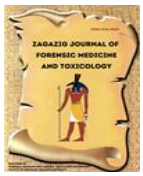

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Original article

Differentiating Drowning from Postmortem Submersion in Freshwater with Estimation of the Postmortem Submersion Interval in Albino Rats

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Abstract

Background: Examining drowning-related fatalities remains a significant challenge for forensic experts in its diagnosis worldwide. Furthermore, techniques for calculating the postmortem interval (PMI) are not relevant to the remains recovered from water because of the intrinsic distinctions between terrestrial and aquatic systems, hence we need to allocate new methods to estimate postmortem submersion interval (PMSI) in bodies recovered from water. **Aim**: To develop reliable forensic methodologies for distinguishing between antemortem drowning and postmortem submersion in freshwater environments while establishing accurate techniques for estimating the duration of postmortem submersion intervals. **Methods:** One hundred adult male albino rats were allocated into two groups: Group 1, rats drowned in freshwater until death, and Group 2, euthanized via cervical dislocation and thereafter submerged in water. Each group was subdivided into five subgroups where rats were dissected at 0, 12, 24, 48, and 72 hours PM. Afterwards, the lungs of rats were dissected and processed for histopathological and morphometric studies.

Results: Apoptotic cells and degenerated areas were significantly more abundant in drowned rats than in postmortem submersed rats, with significant differences observed with PMSI in each group. Both apoptotic cells and degenerated areas showed a strong, and significant correlation with PMSI in both groups, demonstrating high to moderate reliability in PMSI estimation using simple regression analysis.

Conclusion: Apoptotic cells and degenerated areas are valuable for diagnosing drowning and estimating PMSI

I. Background

Drowning ranks as the third leading cause of unintentional injury fatalities worldwide in forensic medicine. Drowning refers to death caused by the infiltration of liquid into the airways due to submersion. Medico-legal investigations of bodies recovered from aquatic environments present significant challenges for forensic pathologists (Ojanperä & Kriikku, 2024).

Traditional drowning diagnosis relies on a combination of macroscopic findings, such as foam in the airways, water in the stomach, pulmonary edema, and emphysema, along with histological evidence, including alveolar distension and intraalveolar hemorrhage. However, these findings are often non-specific, and additional testing methods may have significant limitations regarding sensitivity and specificity (Marella et al., 2019). Additionally, biochemical markers such as electrolyte imbalances may provide supportive evidence but are insufficient as standalone diagnostic criteria (Armstrong & Erskine, 2018).

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Electrolyte imbalances, water aspiration or ingestion, vomiting, the diving response, and the fear of drowning are all components of underwater physiology. Examinations of the heart, lungs, and brain revealed how submersion affects the physiological outcome (Stephenson & Byard, 2023).

A critical challenge in drowning investigations is determining the postmortem submersion interval (PMSI), which represents the time elapsed between body immersion in water and retrieval (Zhang et al., 2024). Current PMSI estimation techniques rely on subjective assessment of decomposition stages, including skin slippage, marbling, and bloating patterns. Quantitative methods have shown promise but demonstrate limited accuracy in aquatic environments due to variable temperature gradients and water movements (Palazzo et al., 2020).

The complexity of PMSI determination arises from the distinct decomposition phenomena and patterns exhibited by the corpses submerged in water, resulting from the interaction of the cadaver and the aquatic environment (Hui-Ya et al., 2020). Traditional taphonomic methods developed for terrestrial environments often prove inadequate when applied to aquatic settings, creating a significant gap in forensic methodology.

Aquatic conditions, such as algae growth, adipocere development, and water composition, alter the pace of postmortem degradation. The nature of the submersing fluid and its temperature are significant factors in decomposition in water (Palazzo et al., 2020). Despite numerous studies exploring aquatic decomposition, there remains a lack of standardized and reliable methods for accurate PMSI determination across different aquatic environments.

Therefore, this study aims to differentiate drowning in fresh water from postmortem submersion with PMSI estimation.

II. Material and Methods

This research was carried out in the forensic medicine and toxicology department, faculty of medicine, Minia University.

The research was authorized by Minia University's Animal Use and Care Committee, permission No: 678-9/2020

II.1 Study design

This experimental comparative study aimed to ascertain and compare the postmortem interval (PMI) following drowning and postmortem submersion. The experiment followed a factorial design with two main groups (drowning and postmortem submersion) and five time points (0, 12, 24, 48, and 72 hours).

Sample Size and Selection

One hundred mature male albino rats weighing 250-350 grams were used in this study. The specimens were acquired from the Laboratory Animal Breeding Facility at Minia University in Minia, Egypt. This sample size was determined to ensure adequate statistical power for between-group comparisons, with ten animals per subgroup allowing for reliable histological assessment and statistical analysis.

During the experiment, the animals were kept in clean, well-ventilated plastic cages at a constant temperature of 20 ± 5 °C. All rats received a balanced diet of standard pellets and water from the tap.

II.2 Methods

Water Sample Collection

The rats were placed in a 0.5 × 1.5 × 0.5 meters basin containing 50 liters of tap water, maintained at 20 ± 5 degrees Celsius.

Experimental Groups

The animals were divided into two main groups:

Group 1 (Drowning)

Fifty rats were split into five subgroups of ten each:

- **Group (1-0hr):** Rats were fully conscious and drowned by submersion in a water basin until death, then removed after being submerged for 10 minutes (Paulis and Hasan, 2018).
- **Group (1-12hr):** Rats were removed from the water after 12 hours.
- **Group (1-24hr):** Rats were removed from the water after 24 hours.
- **Group (1-48hr):** Rats were removed from the water after 48 hours.
- **Group (1-72hr):** Rats were removed from the water after 72 hours.

Group 2 (Postmortem Submersion)

Fifty rats were split into five subgroups of ten each:

- **Group (2-0hr):** Rats were anesthetized with alfaxan 1.5 mL/kg intravenously, then sacrificed by cervical dislocation, and left in water for 10 minutes (Lee et al., 2019).
- **Group (2-12hr):** Rats were left in water for 12 hours.

- **Group (2-24hr):** Rats were left in water for 24 hours.
- **Group (2-48hr):** Rats were left in water for 48 hours.
- **Group (2-72hr):** Rats were left in water for 72 hours.

In both groups, small weights were attached to the carcass (typically to hindlimbs) to prevent floating.

The selected time points (0, 12, 24, 48, and 72 hours) were chosen based on previous forensic studies investigating postmortem changes in drowning cases (Byard et al., 2002). These specific durations allowed for observation of early (0-12h), intermediate (24h), and late (48-72h) postmortem changes in pulmonary tissue, which have been established as critical periods for forensic time-of-death determination in drowning cases. Additionally, this timeline aligned with practical forensic investigation timeframes encountered in real-world scenarios.

Autopsy and Tissue Collection

All rats were autopsied, and lung tissues were collected for histological investigation.

Histopathological Study

Lung tissue specimens were fixed in 10% formal saline for 24 hours and processed to make paraffin blocks. Thin slices measuring 5 micrometers (μm) were cut from these blocks and stained with hematoxylin and eosin (H&E) according to standard protocols (Suvarna et al., 2013).

Morphometric Analysis

Two factors were semi-quantitatively assessed using morphometric analysis by an observer blinded to the experimental groups. At a magnification of 400x, all parameters were measured in 10 separate, non-overlapping regions for every animal using Image J software (Ahmed et al., 2020).

The parameters measured were:

1. The mean quantity of apoptotic cells (those with very dark cytoplasm and small, dense, irregular nuclei) in lung tissues across all ten parts.
2. The mean number of degenerated areas in each of the ten fields.

II.3 Data analysis

All data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Results were expressed as mean ± standard deviation (SD). Comparisons between the drowning and postmortem submersion groups at each time point were performed using a One-way analysis of variance (ANOVA). Post-hoc Tukey's test was employed to identify specific differences between subgroups when ANOVA detected a significant main effect. Tukey's test was specifically selected due to its ability to control Type I errors when making multiple pairwise comparisons while maintaining appropriate statistical power compared to more conservative approaches (McDonald, 2014). A p-value <0.05 was considered statistically significant for all analyses.

The parameters were as follows:

1- The mean quantity of apoptotic cells (those with very dark cytoplasm and small, dense, irregular nuclei) in lung tissues across all ten parts.

2- The mean number of degenerated areas in each of the ten fields.

III. Results

Histopathological results

Hematoxylin and eosin (H&E):

Group 1 (drowning):

Group (1-0hr) showed alveolar sac dilatation, thick septate containing inflammatory cells, edema, and interalveolar hemorrhage. In comparison, group (1-12hr) exhibited macrophages containing brownish hemosiderin droplets, hemorrhage, and thick interalveolar septa. Some alveoli appeared dilated, while others appeared collapsed. Lobular distortion with dilated congested blood vessels was noticed in group (1-24hr). Examination of group (1-48hr) showed a dilated alveolar sac with dilated congested blood vessels and severely thickened interalveolar septa, while group (1-72hr) showed massive lung distortion and degeneration, (Figure 1).

Group 2 (post-mortem submersion):

Group (2-0hr) displayed thick septate and dilated blood vessels, while group (2-12hr) showed thick interalveolar septa and alveolar sac dilatation. Group (2-24hr) showed thick interalveolar septa with multiple degenerated areas. Group (2-48hr) also showed a dilated alveolar sac with distorted lung architecture, while group (2-72hr) showed massive lung distortion and degeneration, (Figure 2).

The mean number of apoptotic cells:

In the drowning group, the mean number of apoptotic cells revealed a significant variation across the different PMSI, as well as between each two PMSI of the drowning group (Figure 3).

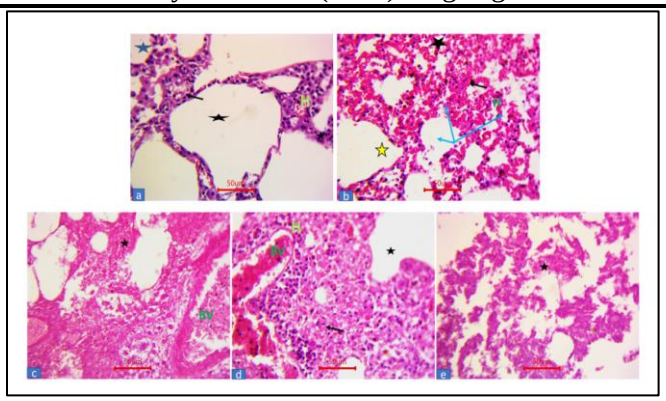

Figure (1): Representative photomicrographs of sections of a rat lung of drowning group: a) Group (1-0hr) showing alveolar sac dilatation (black star), thick septate (black arrow) containing inflammatory cells, edema (blue star), and interalveolar hemorrhage (H). b) Group (1-12hr) showing macrophages containing brownish hemosiderin droplets (blue arrow), hemorrhage (H), and thick interalveolar septa (black arrow). Notice that some alveoli appear dilatation (yellow star) while others appear narrow (black star). C) Group (1-24hr) showing lobular distortion (black star) with dilated congested blood vessels (BV). d) Group (1-48hr) showing dilated alveolar sac (black star) with dilated congested blood vessels (BV). Notice severe thickened interalveolar septa (black arrow). e) Group (1-72hr) showing massive lung distortion and degeneration (star). H&E X 400.

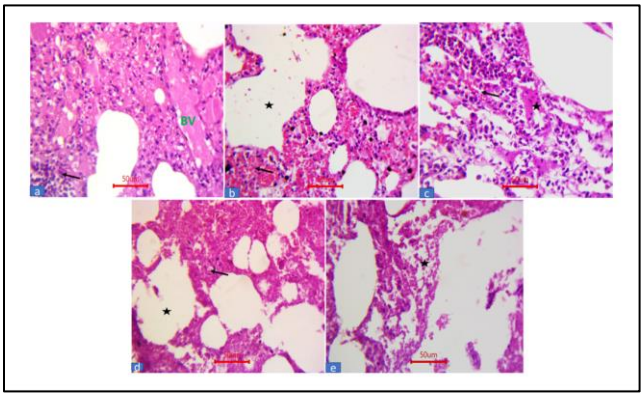

Figure (2): Representative photomicrographs of sections of a rat lung of the postmortem submersion group: a) Group (2-0hr) showing thick septate (black arrow) and dilated blood vessels (BV). b) Group (2-12hr) showing thick interalveolar septa (black arrow) and alveolar sac dilatation (star). c) Group (2-24hr) showing thick interalveolar septa (black arrow) with degenerated area (star). d) Group (2-48hr) showing dilated alveolar sac (black star) with distorted lung architecture (black arrow). e) Group (2-72hr) showing massive lung distortion and degeneration (star). H&E X 400.

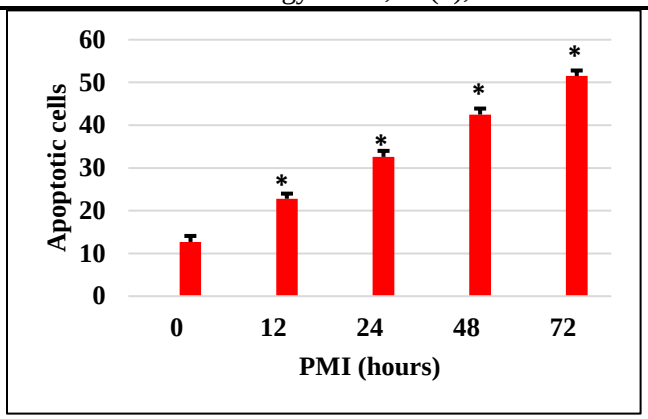

Figure 3: The mean number of apoptotic cells at different PMSI of the drowning group *: Significant difference in comparison to (0 hr.)

The mean number of apoptotic cells in the postmortem submersion group. It shows a significant variation across different PMSIs and between each of the two PMSIs of the postmortem submersion group (Figure 4). In addition, the mean number of apoptotic cells significantly increased in drowning than in postmortem submersion groups at all PMSI (from 0 hr to 72 hr), (Table 1).

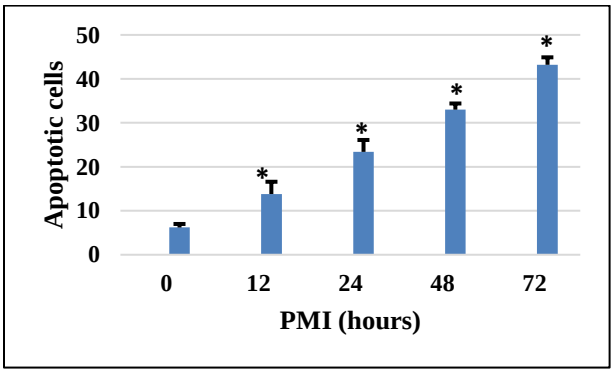

Figure 4: The mean number of apoptotic cells at different PMSI of the submersion group, *: Significant difference in comparison to (0 hr.)

The mean number of degenerated areas:

The mean number of degenerated areas in the drowning group differed significantly across the various PMSI. There was a significant difference between each two PMSI, while insignificant differences were revealed between 0 hr. & 12 hr. and between 12 hr. & 24 hr. (Figure 5).

Table (1): Independent Samples T-test for the mean number of apoptotic cells between drowning and postmortem submersion groups

Apoptotic cells		Drowning	Post-mortem submersion	P value
		N=10	N=10	
0 hr	Range Mean ± SD	(10-14) 12.7±1.4	(5-7) 6.2±0.8	<0.001*
12 hr	Range Mean ± SD	(21-25) 22.8±1.2	(10-18) 13.8±2.8	<0.001*
24 hr	Range Mean ± SD	(31-35) 32.6±1.4	(20-28) 23.4±2.7	<0.001*
48 hr	Range Mean ± SD	(41-45) 42.5±1.4	(31-35) 33±1.4	<0.001*
72 hr	Range Mean ± SD	(50-54) 51.5±1.3	(40-45) 43.2±1.7	<0.001*

*: Significant level at P value < 0.05, SD: standard deviation N = number, hr: hour

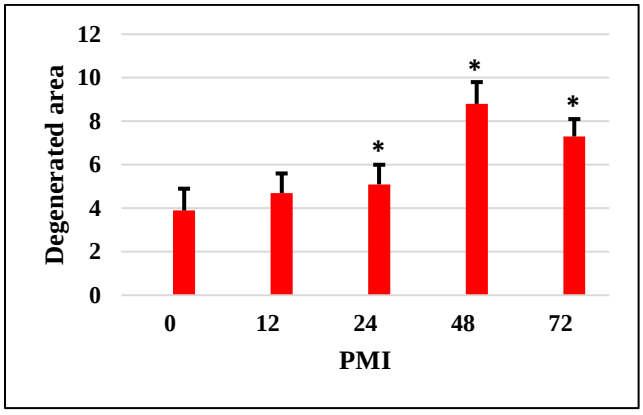

Figure 5: The mean number of degenerated areas at different PMSI in the drowning group, *: Significant difference in comparison to (0 hr.)

The mean number of degenerated areas in the postmortem submersion group revealed a significant difference between different PMSI and between each PMSI except between 24hr & 48hr. (Figure 6)

Table (2) shows that the decrease in the mean number of degenerated areas was significantly greater in the drowning group compared to the postmortem submersion group across all PMI (from 0 to 72 hr.)

In the group that drowned, there was a positive correlation between the mean number of apoptotic cells and degraded regions with PMI., as shown in (Table 3).

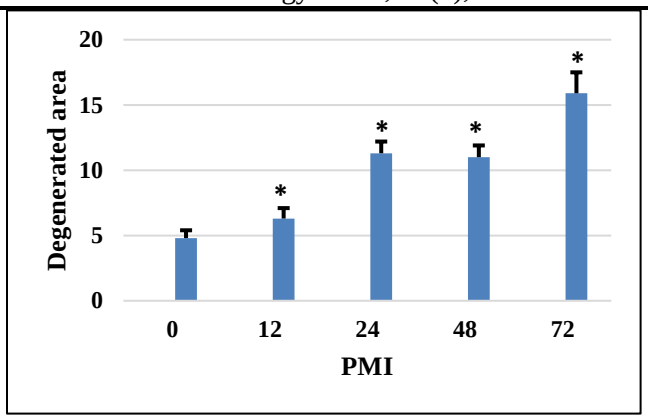

Figure 6: The mean number of degenerated areas at different PMSI in the submersion group, *: Significant difference in comparison to (0 hr.)

Table (2): Independent Samples T-test for the mean number of degenerated areas between drowning and postmortem submersion groups

Degenerated area		Drowning	Post-mortem submersion	*p* value
		N=10	N=10	
0 hr	Range Mean ± SD	(2-5) 3.9±1	(4-6) 4.8±0.6	0.027*
12 hr	Range Mean ± SD	(3-6) 4.7±0.9	(5-7) 6.3±0.8	0.001*
24 hr	Range Mean ± SD	(4-6) 5.1±0.9	(10-13) 11.3±0.9	<0.001*
48 hr	Range Mean ± SD	(7-10) 8.8±1	(10-12) 11±0.9	<0.001*
72 hr	Range Mean ± SD	(6-9) 7.3±0.8	(13-18) 15.9±1.6	<0.001*

*: Significant level at P value < 0.05, SD: standard deviation, N= number, hr: hour

Table (3): Pearson's correlation of the percentage of the mean number of apoptotic cells and the mean number of degenerated areas with PMSI in the drowning group

Parameters	PMSI	
	r	P value
Apoptotic cells	0.978	<0.001*
Degenerated area	0.755	<0.001*

*: Significant level at P value < 0.05, r: Pearson's correlation coefficient

Table (4) shows that when predicting PMSI in groups of drowning victims, simple linear regression analysis indicated that the mean number of apoptotic cells was highly reliable (R2=0.955), while the mean number of degraded regions was moderately reliable (R2=0.561). These findings suggest that it may be possible to estimate PMSI using basic linear regression analysis. The equation for the regression is PMSI = constant + (B * independent variable).

Table (4): Simple linear regression analysis of the mean number of apoptotic cells and the mean number of degenerated areas in the drowning group

	Unstandardized coefficients		*p* value	Adjusted R^2	SEE	Regression equation
	Constant	B				
Apoptotic cells	-28.06	1.83	<0.001*	0.955	5.56	PMSI= -28.06 + (1.83 x Apoptotic cells)
Degenerated area	-26.39	9.66	<0.001*	0.561	17.3	PMSI= -26.39 + (9.66 x egenerated area)

*: Significant level at P value < 0.05, SEE: Standard error of estimate, Regression equation: PMI = constant + (B * independent variable), R2: coefficient of determination

There was a strongly significant positive association between PMSI and the mean number of degenerated areas and apoptotic cells in the postmortem submersion group. (Table 5).

Table (6) shows that the postmortem submersion group's mean apoptotic cell count (R2=0.961) and mean degenerated area count (R2=0.832) were reliable in predicting PMSI using simple linear regression analysis. Such findings suggest that it may be possible to estimate PMSI using basic linear regression analysis. The equation for the regression is PMSI = constant + (B * independent variable).

Table (5): Pearson's correlation of the mean number of apoptotic cells and the mean number of degenerated areas with PMSI in the postmortem submersion group

	PMI	
	R	P value
Apoptotic cells	0.980	<0.001*
Degenerated area	0.914	<0.001*

*: Significant level at P value < 0.05, r: Pearson's correlation coefficient

Table (6): Simple linear regression analysis of the percentage of the mean number of apoptotic cells and the mean number of degenerated areas in the postmortem submersion group

	Unstandardized coefficients		*p* value	Adjusted R^2	SEE	Regression equation
	Constant	B				
Apoptotic cells	-14.26	1.9	<0.001*	0.961	5.17	PMI= -14.26 + (1.9 x Apoptotic cells)
Degenerated area	-25.99	5.8	<0.001*	0.832	10.69	PMI= -25.99 + (5.8 x Degenerated area)

*: Significant level at P value < 0.05, SEE: Standard error of estimate, Regression equation: PMSI = constant + (B * independent variable), R^2: coefficient of determination

IV. Discussion

Accurately differentiating between drowning and postmortem immersion in corpses found in water remains a significant challenge in forensic practice. This issue becomes even more complex when the corpse is significantly decomposed with increased postmortem submersion interval (Xiong et al. 2021).

Hence, this study was designed to differentiate freshwater drowning from postmortem submersion with PMSI estimation.

Our results regarding H&E staining of the drowning group were quite similar to those observed in the postmortem submersion group in the form of thick septate, dilated blood vessels, and dilated alveolar sac till lung distortion and degeneration at 72 h PM. In addition, edema, hemorrhage, and inflammatory cells were evident in drowning group.

These findings result from the influx of freshwater, which traverses the alveolar-capillary barrier into microcirculation and induces alterations in surfactants, causing atelectasis, intrapulmonary shunts, reduced surface tension, and ventilation/perfusion mismatch. Hypervolemia, pulmonary vasoconstriction, and changes in pulmonary capillary permeability all contributed to the worsening of pulmonary edema in the deceased (Barranco et al., 2019).

Classic findings in freshwater drowning include "emphysema aquosum" (overdistended, fluid-filled alveoli) and pulmonary edema. For example, Fornes et al. (1998) reported that computer-aided lung morphometry showed marked alveolar overdistension ("emphysema aquosum") in drowned victims, while alveolar hemorrhage was present but less prominent. Similarly, Paraire (2003) found that fetal and adult drowning cases

often showed capillary congestion and frothy edema filling the alveoli.

Additionally, Suresh et al. (2024) "An Indian autopsy series" found pulmonary congestion (92% of cases) and edema (96%), with alveolar dilation in ~86% and thinning or rupture of septa in 64–84% of cases. These reports agree that drowning causes acute lung injury: fluid aspirated into airspaces produces edema and "water lung" changes (capillary congestion, septal thinning)

By contrast, Adel et al. (2024) stated that postmortem submersion (body placed in water after death) typically lacked these changes. Without breathing effort, the lungs of a submerged cadaver often showed only passive fluid entry; alveoli tended to remain relatively collapsed and showed minimal edema or emphysema. Additionally, Frisoni et al. (2022) reported that in practice, no single microscopic feature (such as intra-alveolar hemorrhage, emphysema, or fluid) uniquely identified drowning; all had to be interpreted within context.

Furthermore, this study revealed that the mean number of apoptotic cells had a significant, strong positive correlation with PMI in drowning and postmortem submersion groups. Simple linear regression analysis for predicting PMI showed higher reliability of the mean number of apoptotic cells in the postmortem submersion group (R2=0.961), followed by the downing group (R2=0.955).

Apoptotic cells as PMSI biomarkers. In recent years, some studies have proposed quantifying lung apoptosis to estimate the submersion interval. The underlying idea is that viable drowning induces apoptosis (e.g., from hypoxia/oxidative stress), whereas a corpse does not mount a similar response. Azouz et al. (2025), using a rat model, (rat model) found strong caspase- 3 immunoreactivity in alveolar cells after drowning, whereas and only faint staining was observed after postmortem immersion. Quantitatively, Adel et al. (2024) reported that drowned lungs had a much larger fraction of caspase-positive area than postmortem-submerged lungs. This suggests that the apoptotic index can differentiate drowning from PMS.

The pathophysiology of ALI is directly associated with the apoptosis of lung epithelial cells. Numerous studies have demonstrated that cytokines and inflammatory cells can cause cell death (Martin et al., 2003 & Chopra et al., 2009). This is the reason why the mean number of apoptotic cells was significantly higher in the drowning group than in the postmortem submersion group.

More broadly, apoptotic markers correlate with postmortem interval in other tissues. For example, Dorandeu & de la Grandmaison (2013) used the TUNEL assay on rat skin and showed the fraction of apoptotic cells rose over 0–48 h and was statistically correlated with PMI. Similarly, Xie et al. (2024) in mouse skin Bax and cleaved caspase 3 protein levels peaked at ~8–12 h postmortem (Bcl 2 peaked by ~24 h). These findings imply a time-dependent increase in apoptotic signaling after death.

By analogy, lung tissue in drowning cases may accrue apoptotic signals with time. Indeed, the Azouz et al. (2025) study's mRNA data showed that caspase 3 expression increased with time (24–48 h) after drowning.

Also, Dorandeu & de la Grandmaison (2013) demonstrated that the apoptosis of skin cells was related to the postmortem processes. In the early postmortem period (less than 48 hours after death), the apoptosis rate was significantly associated with the postmortem interval. However, no published regression models specifically link lung apoptosis to PMSI. Existing studies are preliminary, with small animal samples and limited time points.

In the current study of the drowning group and postmortem submersion group, the mean number of degenerated areas showed a significant difference between different PMIs. Moreover, the mean number of degenerated areas displayed a significant difference (decrease) between drowning and postmortem submersion groups at all PMI (from 0 hr to 72 hr). In addition, the mean number of degenerated areas had a significant positive correlation with PMI in drowning and postmortem submersion groups. Simple linear regression analysis for predicting PMI showed high reliability of the mean number of degenerated areas in the postmortem submersion group (R2=0.832), followed by moderate reliability in the drowning group (R2=0.561).

This agrees with Zaki et al. (2021) who reported the impact of freshwater drowning on rat lungs at different PMIs (immediately after death, 4hr and 8hr). It was found that drowning induced degenerative changes in the lungs of drowned rats, and as the postmortem interval was prolonged, the severity of these changes markedly increased. These degenerated areas were explained by

apoptosis that occurred during ALI. Augmented or heightened apoptosis may lead to the degeneration of lung tissue (Zaki et al., 2022).

Prolonged postmortem interval in water leads to progressive autolysis and putrefaction of lung tissue. Early in PMSI, the lungs may still show recognizable alveolar structures, but with longer immersion, these structures begin to dissolve. Betz et al. (1993) found that in advanced putrefaction, the lung's alveolar architecture was completely lost, making any histological diagnosis impossible. Similarly, Frisoni et al. (2022) emphasized that after a few days of submersion, decomposition blurred all findings. Consistent with this, quantitative data show that with longer PMI in water, lung weight declines, and pleural fluid increases. For instance, Ishigami et al. (2021) observed that drowning victims with PMI ≥3 days had maximal pleural effusion relative to lung mass.

V. Conclusions and recommendations

It is concluded that both the number of apoptotic cells and degeneration areas are significantly more affected by drowning than postmortem submersion. Therefore, both may be applicable markers for diagnosing drowning. In addition, the number of apoptotic cells could be applied for estimation of PMSI with high reliability and less SEE by using the equation PMSI= -28.06 + (1.83 x Apoptotic cells), as well PMI= -14.26 + (1.9 x Apoptotic cells) in drowning and postmortem submersion consequently. Further research evaluating the applicability of these parameters in cadavers is recommended.

Study limitations

This study was limited to a rodent model and a 72-hour PMSI window. Future research should validate these findings in human tissues and extend the observation period to later decomposition stages.

Declarations

Funding

This study was carried out without any funding.

Ethical approval

This study was approved by Minia University's Animal Use and Care Committee, permission No: 678-9/2020. All experimental procedures followed institutional guidelines for animal research.

Data availability statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declared that there was no conflict of interest.

List of Abbreviations

ALI - Acute Lung Injury

PM - Postmortem

PMI - Postmortem Interval

PMS - Postmortem Submersion

PMSI - Postmortem Submersion Interval

TUNEL - Terminal deoxynucleotidyl transferase dUTP nick end labeling

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