STUDY OF POSSIBLE BIOMARKERS AND ELECTROLYTES FOR DIAGNOSIS OF DEATH IN BOTH FRESH AND SALTWATERDROWNING: A CONTINUAL CHALLENGE IN FORENSIC PRACTICE

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ABSTRACT

The recovery of a corpse from water raises an array of questions with no adequate answer, despite all signs that drowning cases could offer**.** This required other ancillary investigations such as; biochemical changes in different body fluids. **Objectives:** This work aimed at studying biochemical and electrolyte changes to differentiate true drowning from postmortem submersion in fresh and seawater using an experimental drowning model. **Methods:** Five groups of 8 adult male albino rats each were used, including anesthetically mechanically euthanized rats without exposure to submersion (as a control group), Group II: postmortem-submersion (PS) in freshwater, Group III: PS in saltwater, Group IV: truly drowned death (TDD) in freshwater, Group V: TDD in saltwater. **Results:** Certain markers significantly increased in the TDD of the saltwater compared to that of the freshwater group, such as sodium (Na), chloride (Cl), magnesium (Mg), calcium (Ca), tumor necrosis factor-α (TNF-α), creatine phosphokinase (CPK), triglycerides, total protein, albumin, strontium (Sr) and cardiac troponin (pericardial fluid and peripheral blood). The summation of Na+, K+, Cl levels, and Sr concentration significantly increased in TDD of the saltwater group compared to the other groups. On the other hand, some parameters like blood urea nitrogen (BUN), creatinine (Cr), atrial natriuretic peptide (ANP), and surfactant-associated protein (ASP-A) significantly increased in the TDD of the freshwater compared to those of the saltwater group. **Conclusion:** Serum electrolytes and biomarkers could serve as adjunct parameters in confirming drowning and differentiating between freshwater and saltwater drowning as well.

Key Words: drowning; electrolytes; freshwater; saltwater; strontium; postmortem submersion

INTRODUCTION

According to the World Health Organization (WHO), drowning is defined as the development of defacement in the respiratory process following submersion or immersion in fluid **(Piegari et al., 2019).**

Drowning is claimed to be the third foremost reason for death and is responsible for seven percent of injury-linked mortalities. It remains one of the gravest public health concerns where; a total of 360,000 drowning victims during

2015 were recorded by the WHO, excluding victims of natural disasters such as floods, transportation accidents, and deliberate cases either suicidal or homicidal. **(Layon et al., 2009;World Health Organization, 2017).** Egypt has a high incidence of drowning cases because it possesses the Red and Mediterranean seas and the River Nile that represent very long coastlines surrounding and passing through it **(Lin et al, 2015).**

Though the mechanisms of drowning and its related injuries had been presented comprehensively in numerous publications, its identification is still the most challenging task in forensic pathology because cadavers retrieved from an aquatic can or cannot have drowned **(McEwen and Gerdin, 2016).** Systematic autopsy and PM investigations are crucial to distinguish lesions that may verify or revoke drowning as a cause of death. However, PM examination can yield many typical external gross signs and microstructural findings, unluckily,

they are non-specific. Supplementary data such as victims' clinical history, crime scene investigation, witnesses, and physical evidence, are commonly looked for by the forensic examiners to anticipate PM findings and ancillary test results in such situations **(Hyodoh et al., 2016).**

Identification of drowning in fresh- or saltwater yet remains questionable and doubtable. The movement of the drowned bodies in the water could obscure the distinction between both types.Verdict cases of drowning by depending only on pulmonary pathology are frequently demanding since the characteristic edema and congestion are not specific (**Van Beeck et al., 2005).** Autopsy of cadavers found underwater represents a significant percent of medico-legal applications. Yet, the basic query concerning the actual cause of death could not be solved **(Piette and Els, 2006).**

The biochemical profile emerging from true drowning can be differentiated from PM immersion, thanks to multifactorial events such as asphyxia, muscle spasm, pulmonary abnormalities, and voluminous water swallowing. These cascades of incidences cause changes of biochemical parameters that could serve as markers for typical drowning death **(Agoro et al., 2021)**. In forensic practice, there is an urgent need for developing an easy, quick, consistent, and confident technique to ascertain the genuine cause of death when a body is retrieved from water **(Xiong et al., 2021).** So, this study aimed to differentiate between true drowning and PMsubmersion in fresh and saltwater depending on the analysis of certain electrolytes and biochemical markers and evaluate their discriminant capacity individually and jointly.

MATERIALS AND METHODS

Study Design

The study comprised five groups; each of eight adult male albino rats as follows:

- Group I: the control group.
- Group II: postmortem-submersion (PS) in freshwater.
- Group III: PS in saltwater.
- Group IV: truly drowned death (TDD) in freshwater.
- Group V: TDD in saltwater.

All experimental procedures involving animals stringently followed the Animal Welfare research and the Ethics of Animal Use in the Research Committee of Zagazig University, Egypt. Forty adult male Albino rats; (180-220 G of weight) were kept in cages at controlled conditions: (21-24℃ temperature, 50-60% relative humidity, and 12-hour darkness (light-darkness cycle). Animals feed on standard rat chow.

Group, I was only mechanically euthanized under anesthesia. The PS groups were submerged after scarification. Rats of both groups IV and V were drowned inside a box filled with water from the Nile River (freshwater drowning) or filled with water from the Mediterranean Sea (saltwater drowning).

Time passed since the start of the drowning process till the confirmed death was recorded. At autopsy, blood samples from dead rats were collected from the heart and great vessels, centrifuged immediately, and kept at -20℃ **(Elalfy et al., 2019; Agoro et al., 2021).**

Methods

- Serum electrolytes **(**Na, k, Cl, and Mg) were measured **(Barnett et al., 1973; Tietz, 1976a; 1976b; 1983**).
- Biochemical markers (albumin, total protein, creatinine (Cr), creatinine phosphokinase (CPK), and triglyceride) were measured **(Dumas et al., 1971; Ietz 1976; Yatzidis 1987; Fassati and Prencipe, 1982)**. Blood urea nitrogen (BUN) was estimated with a urease-glutamate dehydrogenase technique (**Maeda et al., 2009).**
- Serum Tumor Necrosis Factor-α (TNF-α) was analyzed with (ELISA) (Assay Kit Co, Calif) **(Rui et al., 2009).**
- Quantitative assays of surfactant-associated protein (ASP-A) levels were assayed quantitatively in serum samples that were collected from left and right ventricular blood of all animal groups. An automated enzyme immunoassay system (ELSIA-F300) with kits enclosing two anti-SP-A monoclonal antibodies (SP-A Test F Kokusai; International Reagents Corporation, Kyoto, Japan) was used **(Hino et al., 1994; Zhu et al., 2002).**
- Strontium (Sr) levels were estimated in the serum of rats and the water medium by Zeeman AAS (AAnalyst 600) Perkin-Elmer instrument. The sensitivity, detection limit, and linear range was 0.36, 4.6, and 10–40µg/l, respectively **(Azparren et al., 1994; Pérez-Cárceles et al., 2012)**.
- Cardiac Troponin-1: pericardial fluid (PCF) and peripheral blood samples were collected and centrifuged for serum extraction. Cardiac troponin assay was measured by immunoassay, with the "Troponin T hs STAT" assay (Ref. 5092728190, Roche Diagnostics®) **(Zribi et al., 2021)**.
- Also, serum Atrial Natriuretic Peptide (ANP) was measured **(Lorente et al., 1989).**

Statistical analysis

Data was collected and presented as mean ± standard deviation. Data was analyzed using a computer program (SPSS Inc, 2007) version 16.0. A significant difference was calculated with Kruskal–Wallis test, followed by the Post-hoc test for multiple comparisons between different groups. P-values <0.05 were considered significant while values <0.01 and <0.001 were highly significant.

RESULTS

Tables(1 and 2)summarized the serum changes in electrolyte and biochemical parameters of studied rats. Table (3) showed the changes in cardiac troponin levels in both pericardial fluid and peripheral blood. Comparing TDD in the saltwater with that of the freshwater group, there were significant increases in the following parameters Na (mEq/l), K (mEq/l), Chloride (mEq/l), Mg (mg/dl), Ca (mg/dl), TNF-α(ng/L), CPK (mcg/L), Triglycerides (mg/dL), total protein (g/dL), albumin (g/dL), and cardiac troponin (ng/ml) peripheral and central in (both pericardial fluid and peripheral blood). In addition, Na+, K+, and Cl (mEq/L) and Sr(ug/L) levels were significantly increased in the TDD in saltwater compared to other studied groups. On the other hand, TDD of the freshwater showed a significant increase in BUN (mg/dl), Cr (mg/dl), ANP (pg/ml), and A SP-A (Ug/ml)as compared to the saltwater group.

Table 1. Electrolytes and biochemical changes of the serum in different studied groups.

	Control (Group I)	PS in fresh water (Group II)	PS in salt water (Group III)	TDD in fresh water (Group IV)	TDD in salt water (Group V)	p
Na (mEq/l)	135.4±31.2[a,b]	138.1±32.1[a,b]	137.3±30.3[a,b]	109.1 ±16.8[c]	178.8 ±39.9	<0.001*
K (mEq/l)	87.6 ± 19.5[a,b]	89.6 ± 18.8[a,b]	87.6 ± 19.2[a,b]	66.1 ± 11.2[c]	190.1± 35.3	<0.001*
Chloride (mEq/l)	99.3 ± 21.6[a,b]	99.8±20.0[a,b]	100.3±19.1[a,b]	80.1 ±13.3[c]	176.5 ±28.1	<0.001*
SUMNa+K+Cl (mEq/L)*	322.3±24.1[a,b]	327.5±21.9[a,b]	325.2±23.7[a,b]	255.3± 12.6[c]	545.4± 30.6	<0.001*
Mg (mg/dl)	1.7± 0.6[a,b]	2.1±0.7[a,b]	1.9 ±0.8[a,b]	4.5 ±1.6[c]	21.9 ±7.3	<0.001*
Ca (mg/dl)	8.6±2.7[a,b]	8.3 ±1.8[a,b]	9.1 ±2.4[a,b]	10.9 ±2.1[c]	19.0 ±5.6	<0.001*
BUN (mg/dl)	7.4±2.1[a,b]	7.6±2.2[a,b]	8.3±2.1[a,b]	17.4 ±4.1[c]	12.0 ±3.1	<0.001*
Cr (mg/dl)	0.74 ±0.2[a,b]	0.94±0.3[a,b]	0.82±0.2[a,b]	2.8 ±0.9[c]	1.2 ±0.4	<0.001*
TNF-α(ng/L)	13.2 ± 3.4[a,b]	12.9 ± 3.1[a,b]	13.0 ± 2.9[a,b]	11.3 ± 1.4[c]	25.5 ± 6.2	<0.001*
CPK (mcg/L)	498.2±93.2[a,b]	499.4±91.5[a,b]	497.9±94.2[a,b]	402.6±62.1[c]	599.8±99.3	<0.001*
Triglycerides (mg/dL)	91.2±22.3[a,b]	93.5±23.3[a,b]	92.7±21.5[a,b]	68.3±17.1[c]	124.7±31.6	<0.001*
Total protein (g/dL)	8.4±1.5[a,b]	8.7±1.6[a,b]	8.3±1.3[a,b]	6.7±0.9[c]	9.9±1.8	<0.001*
Albumin (g/dL)	3.9± 1.1[a,b]	3.8± 0.9[a,b]	3.9± 1.2[a,b]	2.8± 0.6[c]	4.9± 1.8	<0.001*

* Values of SUMNa+K+Cl are the summation of mean electrolyte concentrations.
Values are expressed as mean ± standard deviation (SD) of n = 8 animals/group
n= number of rats p=0.000 means highly significant
a: P<0.05, compared with GroupIV
b: P<0.05, compared with GroupV
c: P<0.01, compared with GroupV

Table 2. Serum changes of strontium, atrial natriuretic peptide, and surfactant-associated protein changes of the serum in different studied groups .

	Control (Group I)	PS in fresh water (Group II)	PS in salt water (Group III)	TDD in fresh water (Group IV)	TDD in salt water (Group V)	p
Strontium ug/L	24.5±14.8	27.9 ±22.3	28.2 ± 21.8	256.9 ± 569.3	341.6 ±731.4	<0.001*
Atrial Natriuretic Peptide (pg/ml)	147.1±42.5	145.8±39.9	147.3±41.2	358.4 ±96.8	190.3 ±67.2	<0.001*
Surfactant-associated protein (A SP-A) Ug/ml	0.276±0.02	0.259±0.01	0.268±0.02	0.739±0.04	0.491±0.03	<0.001*

Values are expressed as mean ± standard deviation (SD) of n = 8 animals/group
n= number of rats p=0.000 means highly significant
a: P<0.05, compared with GroupIV
b: P<0.05, compared with GroupV
c: P<0.01, compared with GroupV

Table 3. Cardiac troponin level in pericardial fluid and peripheral blood rats indifferent studied groups.

Cardiac troponin (ng/ml)	Control (Group I)	PS in fresh water (Group II)	PS in salt water (Group III)	TDD in fresh water (Group IV)	TDD in salt water (Group V)	p
Pericardial fluid	10.68±2.9	10.79±2.8	11.09±3.1	13.93±4.1	17.91±4.7	<0.001*
Peripheral blood	0.357±0.01	0.357±0.01	0.357±0.01	0.594±0.02	0.851±0.02	<0.001*

Values are expressed as mean ± standard deviation (SD) of n = 8 animals/group
n= number of rats p=0.000 means highly significant
a: P<0.05, compared with GroupIV
b: P<0.05, compared with GroupV
c: P<0.01, compared with GroupV

DISCUSSION

The mystery of determining sure drowning death has remained a major drawback in forensic medico-legal fields, and there is no precise body marker to define drowning. So, incorporating different investigations is significant for

confirming the actual cause of death **(Yajima et al., 2013).**

Various mechanisms expound on the distorted physiological processes that occur due to drowning. The victim is confronted initially with a lack of oxygen and asphyxia as a result of being submerged in water. Alterations that happen include emphysema aquosum and external froth. Blood encounters numerous physical, chemical, and biochemical alterations such as electrolyte imbalance and entry of exogenous substances into the circulation. Insufficiency of accurate morphological alterations in drowned victims raises the need for evaluation via biochemical landmarks **(Paulis and Hasan, 2018).**

The present study aimed to represent and analyze the electrolytes and biochemical alterations to distinguish between drowning death and PM submersion in fresh and seawater. Certain elements significantly increased in rats' serum of TDD in salt- compared to freshwater, such as Na, Cl, Mg, Ca in addition to TNF-α, CPK, triglycerides, total protein, albumin, and cardiac troponin (pericardial fluid and peripheral blood). On the other hand, some parameters like BUN, Cr, surfactant-associated protein, and ANP

were significantly increased in TDD in fresh- compared to saltwater.

The summation of Na+, K+, Cl, and Sr levels significantly rose in TDD in saltwater compared to the other groups. This is considered one of the most significant findings in the current study. These results agreed with several previous studies investigating electrolyte changes in drowning victims. Studies noted that serum levels of Na, Cl, Mg, and Ca rose in saltwater and decreased in freshwater drowning **(Zhu et al., 2003; 2005)**.

On the other hand, other studies reported trivial dissimilarity in blood and CSF regarding Na and K content in freshwater drowning and other causes of death. This inconsistency may be ascribed to delayed PM examination (1–4 days) (**Rammer and Gerdin, 1976)**.

In the current study, we measured the addition of Na, k, and Cl which was consistent with **Yajima and colleagues (2013)**, who stated that using SUM of Na+, K+, and Cl was a preferable diagnostic method when compared to detecting Na+ levels alone or the summation of Na+ and Cl- levels only.

In the same context, **Marella and other researchers (2019)** observed higher levels of Ca and Ma in blood inside the heart and peripheral blood of victims drowned in the sea-as compared to freshwater drowning. Furthermore, they found a high Mg/Ca ratio in pericardial fluid in cases of saltwater drowning.

Pulmonary SP-A is considered a potential indicator for death explication due to hypoxia, mainly drowning. Many experimental studies have reported some surfactant phospholipids as good indicators for differentiating between true and PM drowning and distinguishing between salt- and freshwater drowning **(Zhu et al., 2002).** Other studies showed that pulmonary surfactant decomposed in freshwater but undergone dilution or washing off in saltwater; this could explain our results regarding pulmonary SP-A. However, it may be increased in both blood and tissues, not only in drowned cases but also in other victims of asphyxia fatalities, but to a minimal and insignificant degree. Therefore, higher SP-A levels can be a valuable marker for drowning diagnosis **(Lorente et al., 1990a; Maeda et al., 2003).**

One of the most prominent markers of water aspiration is strontium (Sr), a metal found in a liquid medium rich in it. It is used mainly in differentiation between salt water and fresh water because it is significantly higher in saltwater. Sr is not used alone for the diagnosis of death from drowning. However, it is not present in the same concentration in different rivers. Several researchers assessed its diagnostic efficacy in conjunction with other serum biochemical markers as a beneficial tool in saltwater drowning diagnosis **(Azparren et al., 2003).** Along with our findings, **Pe´rez-Ca´rceles and colleagues (2012)** discovered that drowning is associated with higher Sr blood levels than any other cause of death. Also, they

reported that it is present at an elevated concentration in the sea compared to freshwater.

Atrial natriuretic peptide (ANP) was first experimentally described in a study performed in the year 1990. In parallel with our results, this study found higher blood levels of ANP, especially in freshwater drowning. As a fact, ANP is characterized by its stability in the first eight hours following death. Furthermore, ANP was released physiologically in human volunteers during head-out immersion in water, as well as in all circumstances of hypervolemia **(Lorente et al., 1990b; Piette and Els, 2006).**

In the present study, the significant difference that existed between fresh- and saltwater drowning groups can be referred to the various physio-chemical effects of the drowning boundary, as highlighted by **Keil and colleagues (2014).** Also, **Saukko and Knight (2004),** explained that in freshwater, a large amount of liquid crosses the alveolar membrane and into the circulation, resulting in hemolysis that could liberate K, leading to myocardial anoxia, hypervolemia, K excess, and Na deficit. Anoxia and excess K lead to ventricular fibrillation and death. Conversely in the sea (salt) water drowning, marked hypertonicity of inhaled water causes loss of fluid from the circulation and into the lungs, giving rise to fulminating pulmonary edema with increased blood viscosity leading to circulatory shock and cardiac standstill or asystole, a process that requires 8-12 min to occur (hematocrit and plasma Na level rise).

Moreover, the current study showed significant differences between true drowning and PM submersion groups. This could be attributed to the hypoxia that occurred in both types of drowning, causing depression of the myocardium associated with severe pulmonary dysfunction. Most of these sequels are due to vasoconstriction of pulmonary blood vessels, changes in pulmonary capillary permeability, and associated inflammation, all of which further contribute to pulmonary edema **(Xiong et al, 2021).**

Death by drowning is a major impediment for forensic scientists, not only because of the difficulty in making a conclusive diagnosis for the cause of death but also because it is difficult to assess the circumstances surrounding death. The main difficulty lies in detecting whether the victim died due to drowning, especially in the absence of the morphological signs of drowning or when the body is discovered in a late stage of putrefaction or moved away from water resources **(Legaz et al., 2021)**.

CONCLUSIONS

One of the most challenging and problematic cases in forensic practice is death by drowning. This mandates the development of a more simple and reliable method. The present study suggested the usefulness of conjugation of serum electrolytes and biomarkers for differentiation between true drowning and PM submersion and, also, between fresh- and saltwater drowning.

RECOMMENDATIONS

The present study was conducted in a very early PM time. More studies will be required to convey the biochemical alterations for delayed PM intervals. Also, the correlation between biochemical alterations of the serum and CSF and vitreous, along with the consequences of longer-time submersion, entails further study.

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الملخص العربى

دراسة التغيرات الكيميائية الحيوية والشوارد التي قد تساعد في تشخيص الوفاة الناتجة عن الغرق في المياه العذبة والمالحة: أحد أهم التحديات في مجال الطب الشرعي

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يثير انتشال الجثة من الماء العديد من الأسئلة دون إجابة كافية ، على الرغم من كل الدلائل التى تدل على تشخيص حالات الغرق. و قد نتج عن هذا الحاجة إلى الفحوصات المساعدة الأخرى مثل التغيرات البيوكيميائية و الشوارد في سوائل الجسم المختلفة. يهدف هذا العمل إلى دراسة التغيرات البيوكيميائية و الشوارد من أجل التمييز بين الغرق الحقيقي والغمر بعد الوفاة في المياه العذبة ومياه البحر باستخدام نموذج الغرق التجريبي. تم استخدام خمس مجموعات حيث ان كل مجموعة تتكون من 8 ذكور جرذان بيضاء بالغين:(المجموعة الاولى الضابطة) تم الاعتماد على الموت الرحيم ميكانيكيا للفئران دون التعرض للغمر ،(المجموعة الثانية) فئران ميتة مغمورة في المياه العذبة ، (المجموعة الثالثة) فئران ميتة مغمورة في المياه المالحة، (المجموعة الربعة) فئران حية واعية تمامًا غارقة في المياه العذبة، (المجموعة الخامسة) فئران حية واعية تمامًا غارقة في المياه المالحة. زادت بعض الدلالات بشكل كبير في الوفيات الغارقة حقًا في مجموعة المياه المالحة عند مقارنتها بالموت غرقا في المياه العذبة ، مثل الصوديوم ، الكلوريد، المغنيسيوم ، الكالسيوم ، عامل نخر الورم -الفا) ، فوسفوكيناز الكرياتين ، الدهون الثلاثية ، البروتين الكلي ، الألبومين ، السترونتيوم والتروبونين القلبي (سائل التامور والدم المحيطي). و قد لوحظ ايضا زيادة مجموع كل من الصوديوم والبوتاسيومو الكلورايد وكذلك مستويات السترونشيوم زيادة ذات دلاله احصائية في مجموعة الموت غرقا في المياه المالحة مقارنة بالمجموعات الأخرى. و على الجانب الاخر لوحظ زيادة بعض الدلالات مثل نيتروجين اليوريا في الدم ، والكرياتينين ، والببتيد الأذيني المدر للصوديوم ، والبروتين المرتبط بالسطح بشكل كبير في الفئران الميته غرقا في المياه العذبة مقارنة بتلك االميتة غرقا في الماء المالح. ومن جميع ما سبق فقد وجد ان بعض الشوارد و المؤشرات الحيوية المستخدمة في هذه الدراسة يمكن أن تمثلعوامل مساعدة في تأكيد الغرق كسبب للوفاة و أيضا التمييز بين الغرق في المياه العذبة أوالمياه المالحة.