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Research Article

Receptor for advanced glycation end products (RAGE) as a novel biomarker for differentiating drowning from postmortem submersion

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Abstract

Background and objective: Drowning is the death caused by fluid blocking the airway leading asphyxiation. One of the most difficult challenges in forensic medicine is making the diagnosis of drowning. Drowning is considered one of the most common causes of unnatural deaths worldwide. Determining whether a body entered water before or after it died is crucial in the practice of forensic medicine. The objective of this study was to differentiate drowning from postmortem submersion using receptor for advanced glycation end products (RAGE). **Methods:** Twenty adult male albino rats were used in this study. They were split into 2 groups (drowning and postmortem submersion). There were ten rats per group. Each rat's lungs were meticulously sampled, and PCR was used to assess RAGE expression. **Results:** RAGE expression showed a significant difference between drowning and postmortem submersion groups. **Conclusion:** RAGE could be a helpful biomarker for differentiating drowning from postmortem submersion.

Keywords: Drowning, postmortem submersion, receptor for advanced glycation end products (RAGE).

Introduction

The World Health Organization (WHO) statesthat drowning is one of the top cause s of injury-related fatalities worldwide[1.]

Drowning was defined as the process of experiencing respiratory impairment from submersion in a liquid medium[2]. According to a 2017 analysis by the Global Burden of Disease, 295,210 individuals drowned to death annually on average[3]. Even while the rate was greater in 1998, when approxi-mately 500,000 people died from drowning, the current figures are still worrisome[4].

The events that result in drowning can be divided into the following sequence: (i) fight to keep the airway clear of the water, (ii) initial submersion and breath-holding, (iii) aspiration of water, (iv) unconscious-sness, (v) cardio-respiratory arrest and (vi) death – failure to revive[5].

The physiological causes of hypoxemia are based on the volume and composition of aspirated fluid[6]. Fresh water inhalation reduces the pulmonary surfactant and renders the alveoli unstable, which causes some of the alveoli to collapse and develop atelectasis[7]. With significant pulmonary venous admixture, intrapulmonary shunt occurs. Most of the time, it is not a concern when hypotonic fresh water appears in the alveoli since it is quickly absorbed into the pulmonary and systemic circulation with blood dilution and hypervolemia[8].

90%–95% of the alveolar surface is covered by alveolar type I epithelial cells, which support barrier integrity and alveolar fluid clearance[9]. The receptor for advanced

glycation end products (RAGE) is a transmembrane pattern-recognition receptor of the immunoglobulin superfamily. It is constitutively expressed at low levels in all cells. However, it is abundant in the lung[10].

When examining immersed bodies, forensic pathologists must carefully determine the cause of death and distinguish between drowning and postmortem submersion (the disposal of a body in water after death), as drowning is not necessarily the cause of death. The development of a trustworthy biomarker to distinguish between drowning and postmortem submersion is crucial even though there is no specific biomarker for diagnosing drowning[11, 12].

The objective of this study was to differentiate between drowning and post-mortem submersion using RAGE by RT-PCR.

Material and methods

Animals:

This study used twenty mature male albino rats, weighing between 250 and 350 g apiece. They were acquired from the Minia University's rearing facility for laboratory animals in Minia, Egypt. The animals were kept in plastic cages with free access to balanced standard diet pellets and tap water, as well as good ventilation and hygienic conditions. The experiment was carried out in compliance with Minia University's animal use and care committee's guidelines, approval number 678-9/2020. The months of November and December of 2020 were used for this investigation.

Water sample collection:

A basin of water filled with 12 liters of tab water was used, and the rats were immersed into it at room temperature.

Experimental design:

The rats were divided into 2 groups at random: postmortem submersion (n=10) and drowning (n=10). Rats of drowning group were fully conscious and drowned by submersion of the animal cage in water basin until death[13]. Rats in the postmortem submersion group were anesthetized with ether, sacrificed by cervical dislocation, and submersed in water. The bodies of each rat were autopsied. For RNA isolation, lung samples were collected, and stored at -80°C

RNA extraction, cDNA synthesis and reverse transcription-PCR:

After frozen tissue samples were homogenized in TRI Reagent (1 ml/50 - 100mg tissue) using Polytron homogenizer, the total RNA was extracted using TRI reagent from Molecular Research Center, Inc (Cincinnati, OH, USA) and the tissue protocol was used according to the manufacturer's instructions. The concentration of the extracted RNA was determined using Genova spectrophotometer (Genova Plus, Jenway, Stone, Staffs, UK) and the cDNA synthesis was confirmed using a GoScript™ Reverse Transcription System (PROMEGA Company, USA) according to the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR):

All samples were amplified by real time-PCR using GoTaq® 1-Step RT-qPCR System (PROMEGA Company, USA). The PCR was performed using GoTaq® qPCR Master Mix and primers (Table 1). The PCR reaction mixture included 10 µL of Master mix, 0.25 µL of each primer and 6.5 µL of DNA template in a final volume of 20 µL. The thermal cycling conditions included 1 cycle of Polymerase activation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 5 sec, and 40 cycles of primer Annealing and Extension at 60 °C for 15 se,. The threshold cycle (Ct) was measured and subtracted from CT of housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), to obtain delta CT, and then we subtracted delta CT of diseased case from mean delta CT of control case to calculate delta CT. From these equations, relative quantity (RQ), was calculated $RQ=2^{-\text{delta delta CT}}$. The expression levels of the target mRNA genes were analyzed to determine the ratios of the targets normalized to GAPDH reference, using the ΔΔCT.

Statistical analysis:
Statistical Package for Social Sciences (SPSS) programme version 28 was used to code, tabulate, and statistically analyses the collected data. The mean and standard deviation were used to represent the RAGE expression. The means of the two groups were compared using an independent sample T test. P value of <0.05 was considered significant.

Results

RAGE expression displayed a significant difference between drowning and postmortem submersion groups (P < 0.001) as shown in (table 2, figure 1).

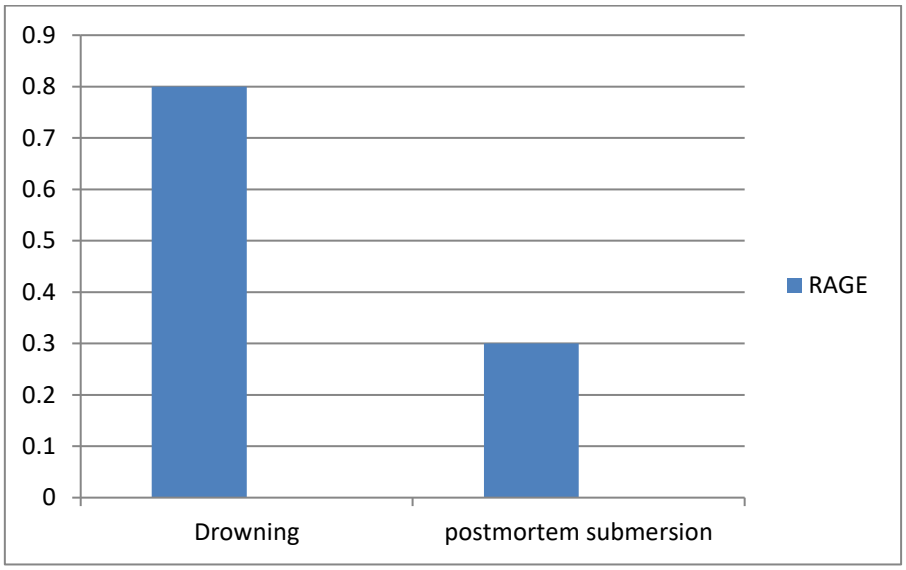

Figure 1: RAGE expression between drowning and postmortem submersion groups

Table (1): Sequence of primers used for quantitative real-time PCR (qPCR)

Gene name	Primer sequence
GAPDH	F: 5′- ATG ACT CTA CCC ACG GCA AG -3′ R: 5′- GAT CTC GCT CCT GGA AGA TG -3′
RAGE	F: 5′- AGC TTC AGT CTG GGC CTT C -3′ R: 5′- CAG CTG AAT GCC CTC TGG -3′

Table (2): Independent Samples T test for parametric quantitative data between drowning and postmortem submersion groups

RAGE (qRT PCR)	Drowning	Postmortem submersion	P value
	N=10	N=10	
Range Mean ± SD	(0.6-1) 0.8±0.1	(0.2-0.5) 0.3±0.1	***<0.001****

- ***SD: standard deviation.***

Discussion

Drowning in fresh water is a relatively common cause of accidental death in both toddlers and adults[14]. Drowning is known as a complex process with several physical reactions, leading to acute respiratory distress syndrome, hypoxic respiratory failure, and cardiorespiratory arrest[15]. Injury to alveolar epithelial cells (AECs), which constitute more than 95% of the interior surface area of the lung, is a crucial characteristic of acute lung injury/acute respiratory distress syndrome (ALI/ ARDS)[16].

RAGE is a transmembrane pattern recognition receptor which expressed in mammals from the immunoglobulin superfamily (Ig)[17]. RAGE is highly expressed in alveolar epithelial cells and serves as a marker of epithelial injury[18]. It`s primary soluble variants, known as soluble RAGE (sRAGE), which lack a transmembrane domain, have good diagnostic value and are linked to the degree of lung injury in clinical studies[19]. The aim of this work was to use RAGE to distinguish between drowning and post-mortem submersion.

To distinguish between drowning and postmortem submersion, some earlier research used lung lesions, serum electrolyte levels, immunohistochemical detection of intrapulmonary surfactant protein A (SP-A) distribution, macrophage levels, and a diatom test[20, 21, 22]. Lee et al., 2017 observed that intra-alveolar granular staining of SP-A was higher in the drowned group than the postmortem submersion and control groups[20].

Our study revealed that RAGE expression had a significant difference between drowning and postmortem submersion groups ($P < 0.001$) which was in agreement with Lee et al study[23], that reported higher expression of RAGE in both seawater drowning ($P < 0.01$) and freshwater drowning ($P < 0.05$) groups than hypoxia, freshwater and seawater postmortem-submersion groups. Thus, it was concluded that RAGE could be a helpful biomarker for differentiating drowning from post-mortem submersion.

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