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Research Article

Pathological Features and Interlukein-6 Expression Distinguishing Recovered Dead Albino Rats after Submersion in Fresh or Saltwater

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Abstract

The investigation of distinguishing the postmortem features of submersion on fresh or salt water is of great concerning in forensic medicine to solve violation in crime and insurance issues. Histopathological features of salt water submersion of rats showed that the brain had a little postmortem change represented by the death of neurons and vacuolation in the brain parenchyma, Heart is showing normal cardiomyocytes and relatively increases in the interstitial spaces and Lung displays pulmonary atelectasis and over distended alveoli with air with death pneumocytes with congestion of interstitial pulmonary capillaries. Histopathological of fresh water submersion of rats revealed that the Brain is showing diffuse neuronal death and vacuolation in the brain parenchyma, heart is showing diffuse death of cardiomyocytes and relatively increasing in the interstitial tissue and lung displays collapsed alveoli, proliferation of interstitial tissue and hemorrhage. Collagen deposition in heart of fresh and salt water submersion is showing blue stained fibrous tissue around the wall of blood vessels and Lung displays blue stained fibrous tissue limited to peribronchial tissue with no interalvolar proliferation.The immune-reactivity of IL-6 recorded at 24 hrs in rats submersed underneath salt water, was recorded in brain > lung>heart displays mild immune-reaction represented by focal brown staining represented by brown staining in the interstitial tissue. While the activity of IL-6 was no or little expression in freshwater submersed and recovered dead rats from freshwater. In conclusion, interleukin-6 expression and postmortem changes could distinguish between salt and fresh water submersion

Key words: salt and fresh water submersion; natural death; collagen; interlukin-6; albino rats

Introduction

The definition of Submersion revealed that the whole body is under water, whereas immersion refers to the body being covered partially with water, although the airway must be immersed for drowning to occur (1).

The identification of the cause of death is very significant in forensic medicine. But after time passed after death, this task become not easy due to morphological and postmortem changes that appeared in rats' cadavers. The differentiation of fresh and salt water submersion is very important in veterinary and human cases and also for insurance purposes.

Detection of the cause of death in animals' cadavers recovered from of water, water-containing vessels or swimming pools, is extreme challenging. Animals recovered from water may or may not evidence of drowning. The diagnosis of drowning is usually need more evidence based information from the crime scene, recovered scene, the medical history and reliable witness accounts. (2, 3,4,5,6).

Submersions of animal bodies were recorded in freshwaterin man-made water sources, natural water sources, and the body of water was not recorded in 1 case. The 71% of submersions cases happen from May through September. The cause of submersion was known in 16 animals including extraordinary circumstances such as falling into water, breaking through ice and intentional submersion. Some animals were found submerged in water with unknown circumstantial evidence (7).

The rationale of this study for investigation the postmortem changes that occurred in submerged cadavers in fresh or salt water.

Material and methods

Animal procedures were conducted with the accordance of the animal care committee of the ethics Board of the faculty of veterinary medicine Mansoura university, Egypt.

24 male Albino rats (weight, 100-150 g; age was purchased from faculty of pharmacy, Mansoura University, Egypt). they were maintained on a 12-h light/dark cycle with free access to food and

water. Rats were exposed to induction of natural death (8,9) then kept enforcedly in wire box underneath in fresh water or salt water for 24 or 48 hrs. after defined time, recover dead rats were desiccated and organs were weighted and then one part fixed in 10 % formalin and the other part preserved in -20C.

Histopathology examination

The tissues like heart, lung, and brain of recovered dead rats form fresh or saltwater were separated at different time point after death and fixed in 10% neutral buffered formalin. Fixed tissue were procedures were described previously before in details for eosin and hematoxylin staining (**10**).

Immunohistochemically Localization of Interleukin-6 (Il-6)

The brain, heart and lung paraffin sections that were mounted on positively charged glass slides and were processed for single enzymatic immunohistochemistry with an antibody to interleukin- 6 (IL-6). This protocol is carried out according to (11).

Morphometrically study:

The mean area percentage of collagen fiber content between the brain, heart and lung of drowned rats either in fresh water or slat water was quantified for each group. The randomly sections stained with Mallory trichrome(x400) (for each group and times) were examined using image J analyzer program. The data that was obtained by this program was statistically analyzed and compared with the each other

Statistical analysis.

Statistical analysis was carried out using the student's t-test and one way annova. $P \leq 0.05$ was considered significant (spss version13) (12).

Results

The distinguishing between salt water and fresh water submersion is very important in forensic medicine. To better understand the pathological and immunological factor that can be used for identification of recovered cadaver from water. In absence of signs of drowning, only gross features will be same in both type of submersion. Notably, brain and lung weight were reduced while heart was increased non significantly in salt water submersed rats when compared to fresh one at 24 hrs. while at 48 hrs. brain and heart weight were reduced, while lung weight has no change in salt water submersed rats when compared to fresh one at 48 hrs. only heart weight has reduced in salt water submersion while lung weight was reduced in fresh water submersion at 48 hrs on time dependent manner.

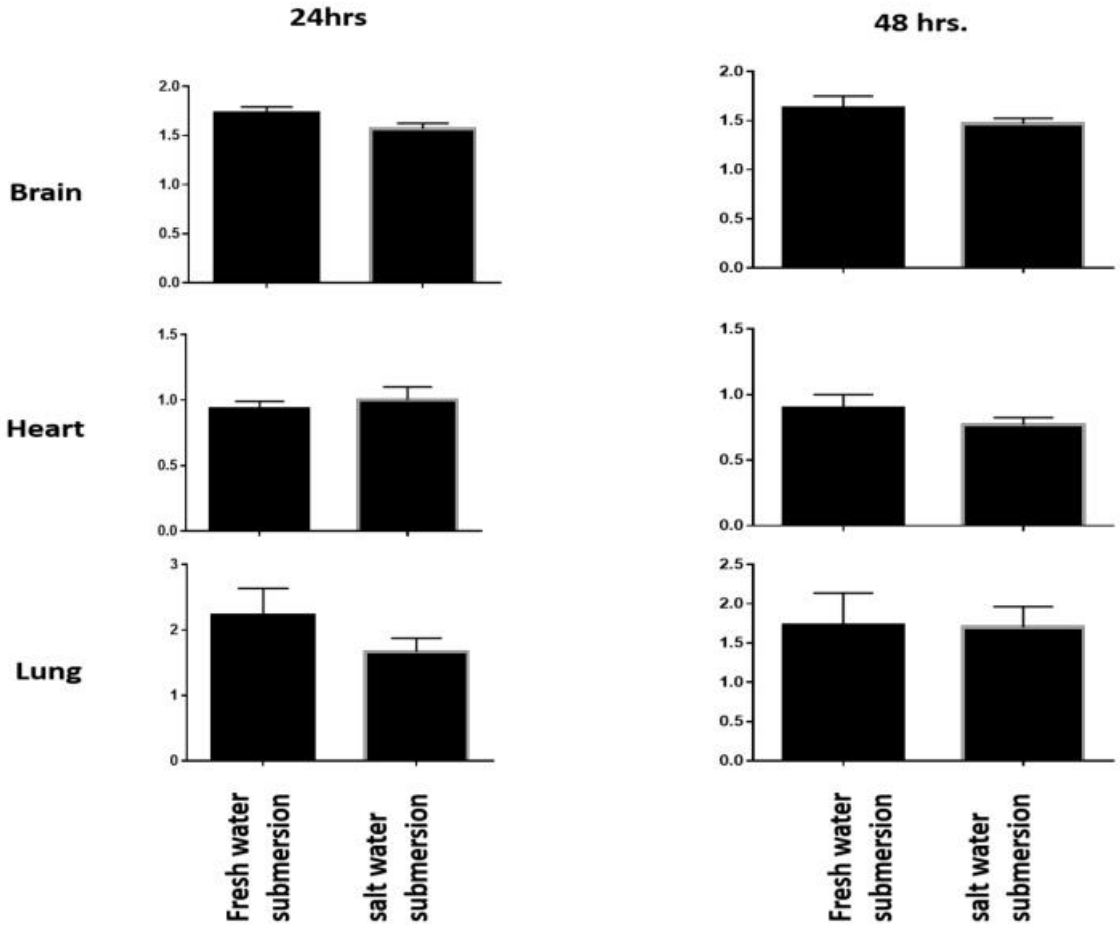

Fig 1. Show that brain and lung weight were reduced while heart was increased non significantly in salt water submersed rats when compared to fresh one at 24 hrs.

While at 48 hrs. brain and heart weight were reduced, while lung weight has no change in salt water submersed rats when compared to fresh one at 48 hrs.

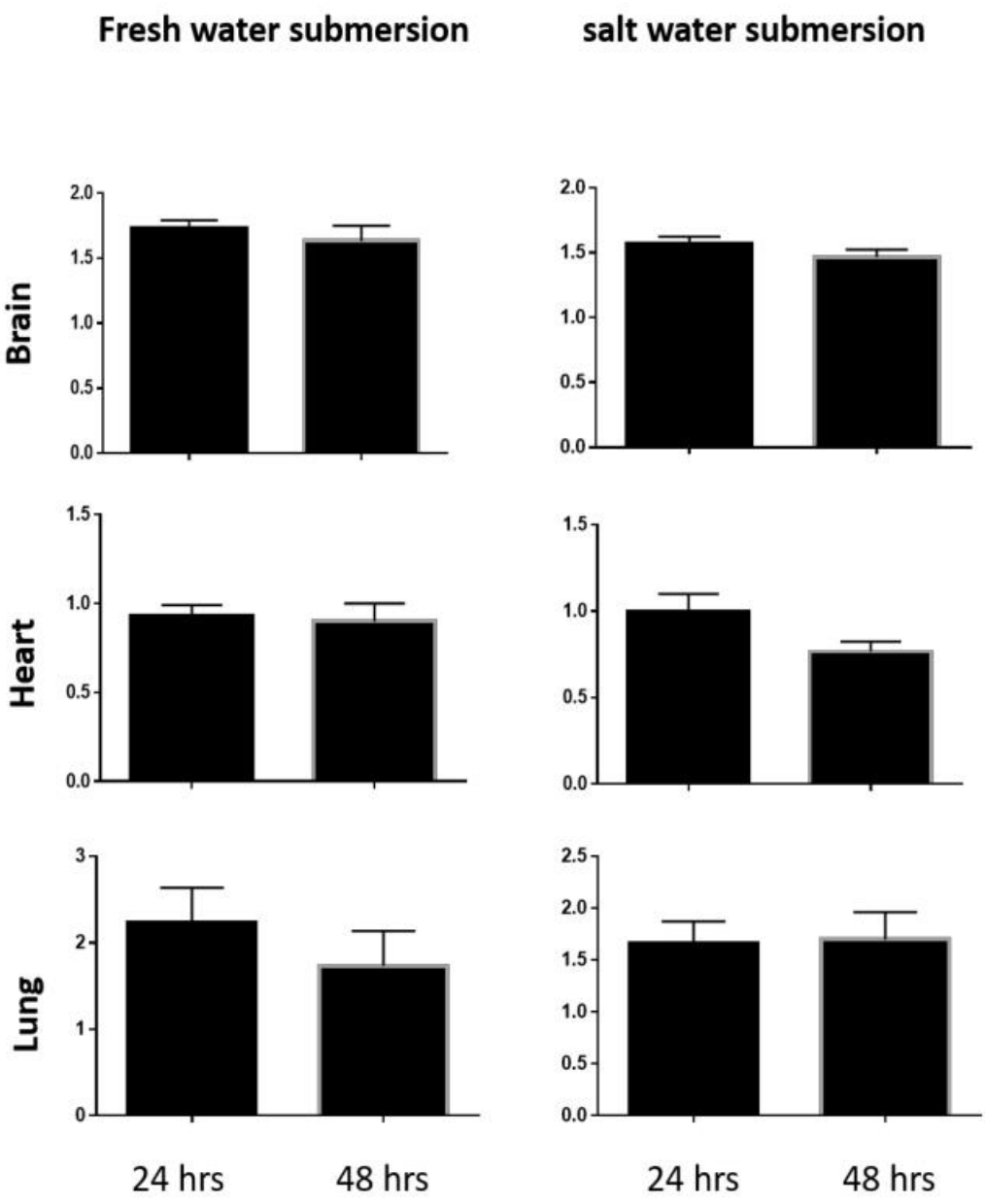

Fig2 show only heart weight has reduced in salt water submersion while lung weight was reduced in fresh water submersion at 48 hrs. on time dependent manner.

Histopathological of fresh water submersion of rats revealed that the Brain is showing diffuse neuronal death and vacuolation in the brain parenchyma, heart is showing diffuse death of cardiomyocytes and relatively increasing in interstitial tissue and lung displays collapsed alveoli, proliferation of interstitial tissue and hemorrhage.

Histopathological features of salt water submersion of rats revealed that the brain is showing postmortem changes represented by death of neurons and vacuolation in the brain parenchyma, Heart is showing normal cardiomyocytes and relatively increases in interstitial spaces and Lung displays pulmonary atelectasis and over distended alveoli with air with death pneumocytes with congestion of interstitial pulmonary capillaries.

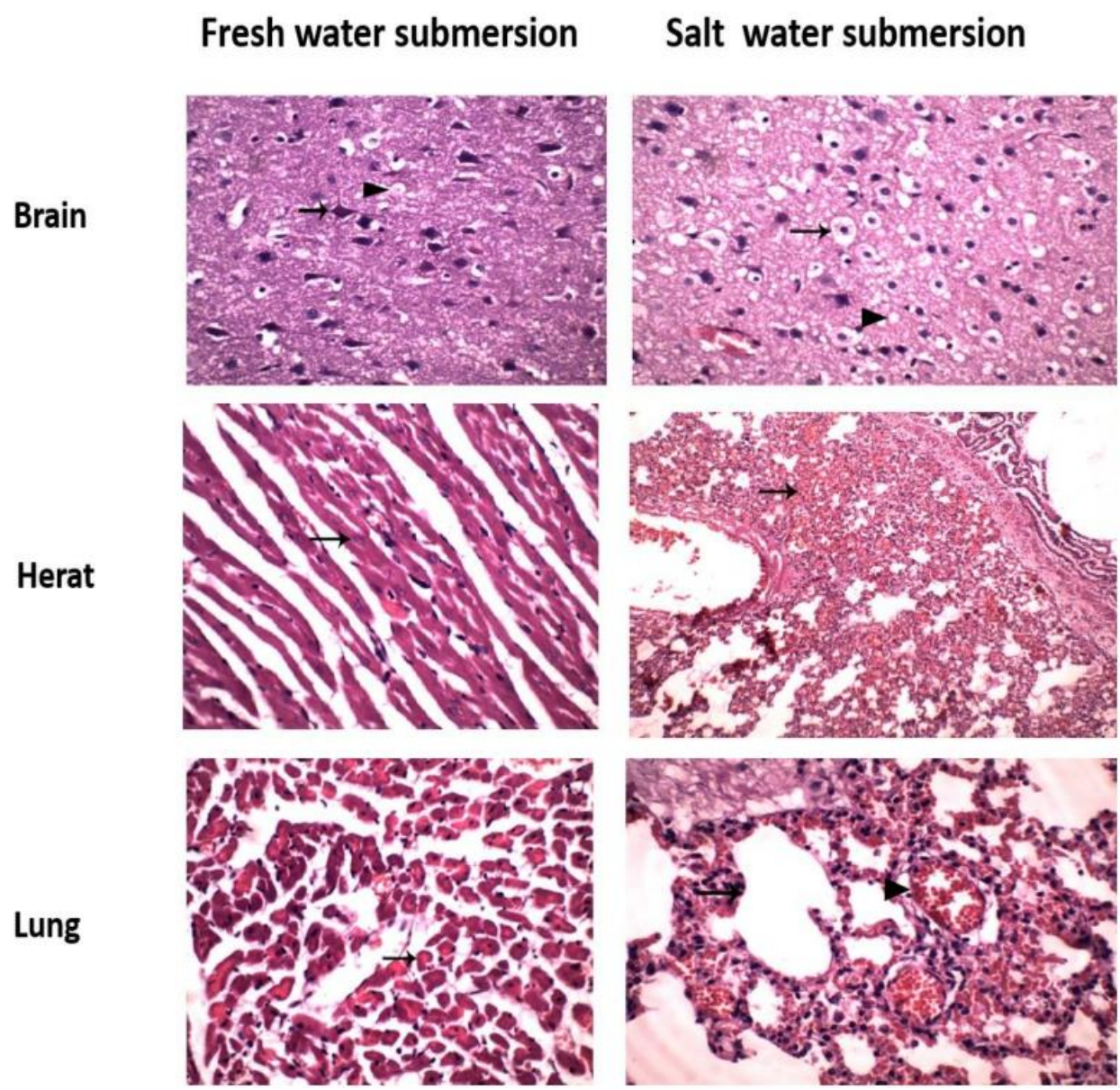

Fig 3; show death of brain, cardiomyocytes and peumocytes with increased edema in lung and hemorrhage in salt water submersion than fresh one.

Collagen deposition in heart of fresh water submersion is showing blue stained fibrous tissue around the wall of blood vessels and Lung displays blue stained fibrous tissue limited to peribronchial tissue with no interalvolar proliferation. Notably, heart of salt water submersed underneath salt water is showing blue stained fibrous tissue around blood vessels in myocardium and lung displays blue stained fibrous tissue around blood vessels with no interalvolar proliferation.

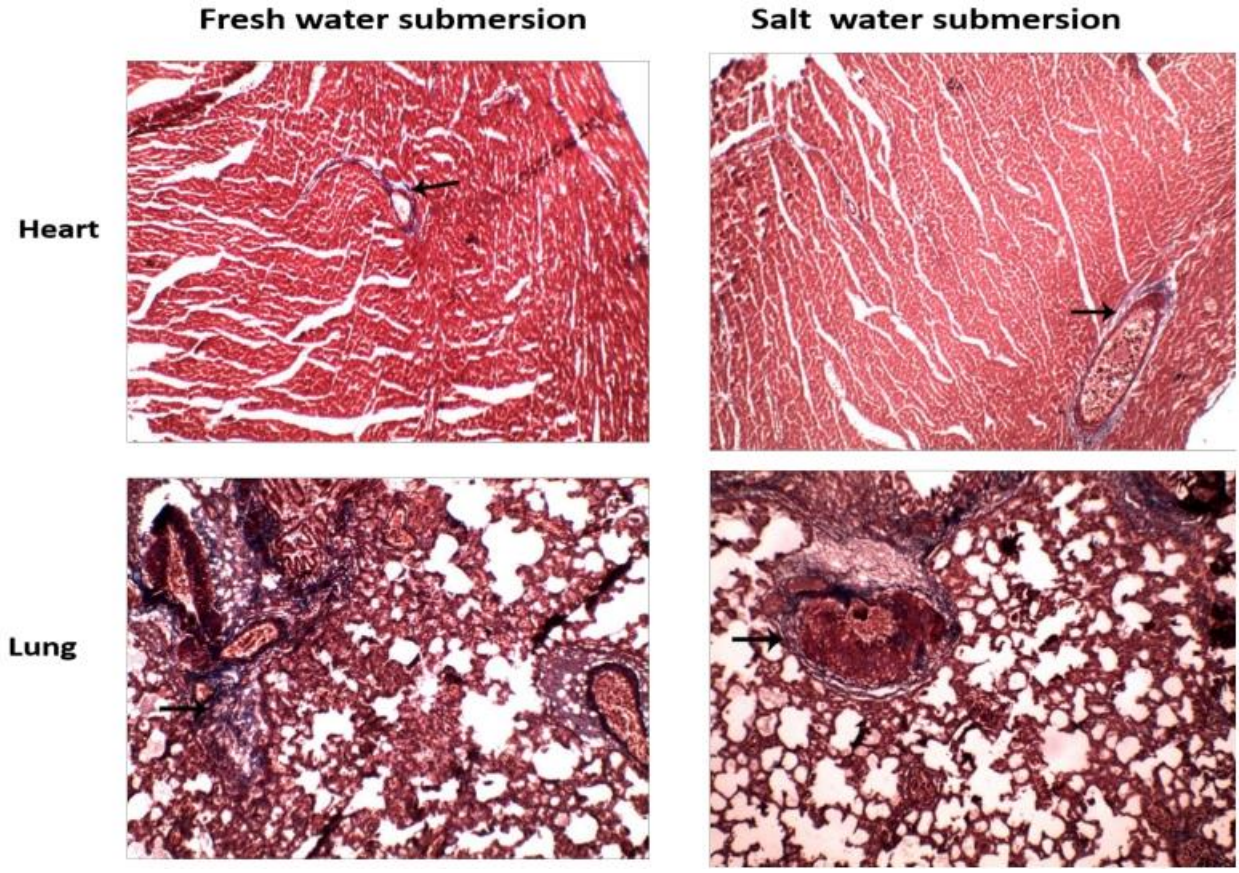

Fig 4. Show that collagen infiltration in heart and lung was more prominent in salt water submersion than freshwater one evidence by trichrome Mallory staining.

The immunoreactivity of il-6 recorded at 24 hrs. in rats submersed underneath salt water, Brain displays moderate Immunoreactivity represented by scattered brown staining in brain parenchyma, heart displays mild Immunoreactivity represented by focal brown staining in cardiomyocytesand lung displays moderate Immunoreactivity represented by brown staining in interstitial tissue.

While the activity of inlukin-6 was little expression in fresh water submersed rats underneath fresh water, Brain displays negative Immunoreactivity represented by absence of brown staining in brain parenchyma, heart displays negative Immuno-reaction represented by absence of brown staining in cardiomyocytes and lung displays moderate reactivity represented by brown staining in interstitial tissue.

Discussion

The frequency of drowning and submersion occur in animals still unknown as most animals can swim and resist drowning, but may be submerged in case of dead or fallen (2). The investigation, identification of circumstantial evidence of submersion of animals in fresh or salt water is very important in forensic medicine. The recorded cases of submerged animals like dog, pig and other animals were recorded even sometimes the cause of death and circumstances couldn't be identified. The significance of that research to differentiate between salt water and fresh water submersion of dead animals to proof violation as it is of high importance for forensic report and medical insurance.

In the current study, we explore pathological, collagen and interleukin-6 activity in submerged rats and its correlation to PMI. As the study of decomposition is essential for any forensic anthropologist for estimating postmortem interval in case of submersion for short or long period. Even surface rates of decomposition have been investigated, especially in certain areas (13), the decaying rate of bodies submerged in water has rarely been studied using controlled research experiments (14,15).

Small animal of low surface area than large livestock animal, relative lack of subcutaneous fat, and their higher surface area to body weight have high incidence ratio similarly to what happen in children [16 and 17). and during submersion, the body was rapid cooling and may protect the brain, allowing delay postmortem changes in brain than other organs (18).

The most prominent pathological changes occurred rapidly in lung and heart while was delayed in the brain in submerged rats due to brain cooling hypothesis may be one accepted explanation for delayed changes in the brain after prolonged submersion. The important factor is how quickly the brain loses the temperature and protect them against hypoxia [17,18]. Similarly, the carotid artery the temperature in dogs anesthetized with thiopental reduced in an accelerating the manner in the first 2 min of both fresh water and

salt water at 4 °C from start of submersion [19]. Even external features and common shared characters between salt and fresh water submersion (20) but here internal pathological identification and cytokines modulation like interlukin-6 could help in this problem issue of differentiation of a victim suffered drowning (21)and other from a body disposed of in water following death (22). Notably, we notice previously that natural brain death had a characteristic feature of pathology in different organs differ than what found in drowning in fresh or saltwater and expression of IL-6 was increased in salt water than freshwater (23).

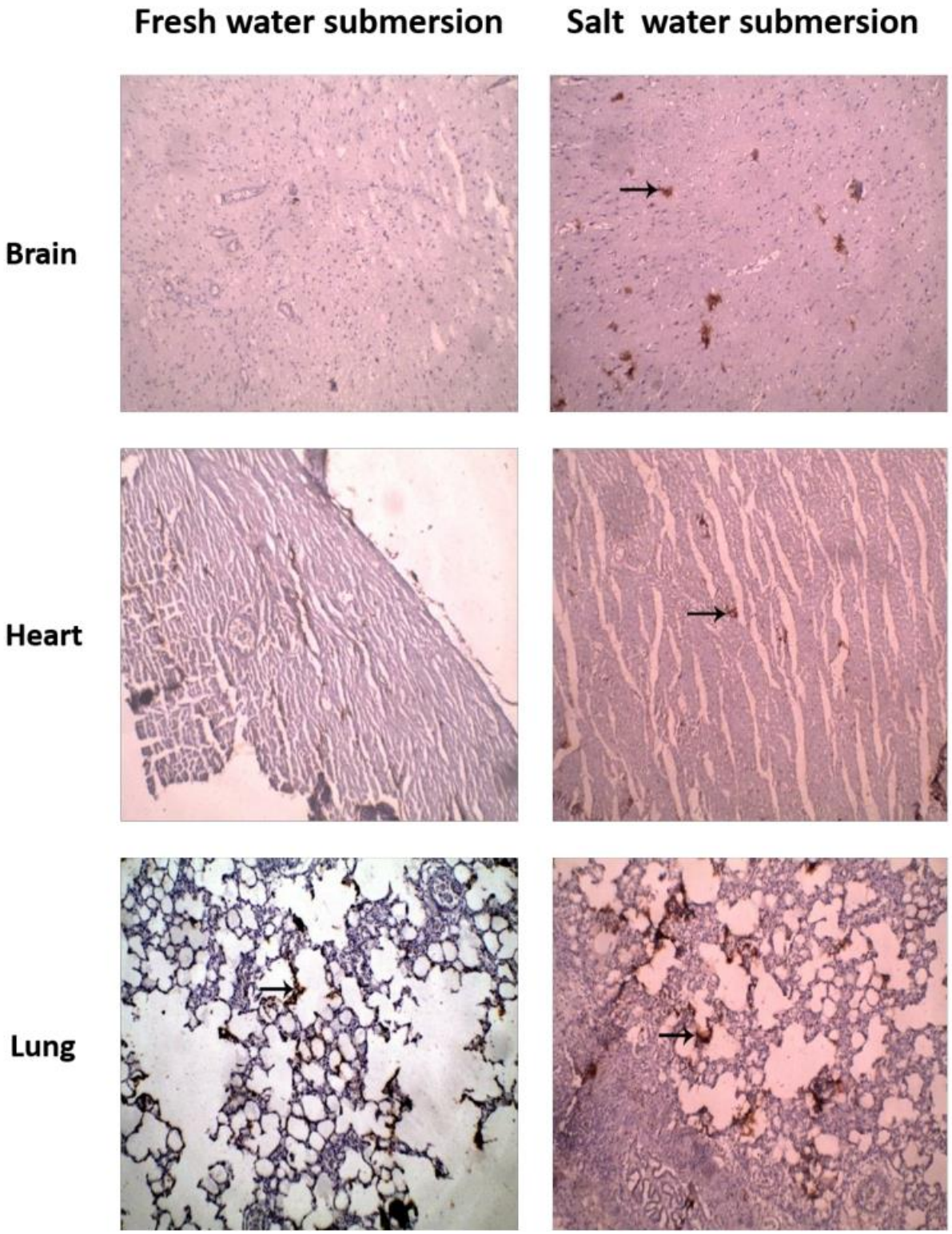

Fig 5. Show that interlukin-6 activity was expressed in heart and lung in more prominent manner in salt water submersion than freshwater one by IHC use hematoxylin as counter staining.

On conclusions, pathological features and cytokines could have distinguished between fresh and salt water submersion. Although this study need further investigation to explain the casualty of immune activity that may be due reaction before or at onset of death.

Conflicts of interest

all authors have no conflict of interest.

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