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STUDY OF BLOOD IRON IN RATS DROWNED IN FRESH AND SALT WATER

Abd El-Wahab A. Dawood *, Afaf M. A. Farghaly *, Soheir A Mohamed, Khaled E Aboul-Hagag, Maha A Hilal, and Rania A Radwan

Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Assuit* and Sohag University

ABSTRACT

BACKGROUND: Postmortem diagnosis of drowning is one of the most difficult tasks in forensic pathology. Autopsy finding may be confused with other types of asphyxia and may not be present during autopsy. **OBJECTIVES**: The aim of the present study was to measure the post-mortem level of iron (*Fe)* in both right and left ventricles of the heart of albino rats and to determine its diagnostic value in differentiation between drowning in fresh and salt-water. **METHODS**: This work was conducted on 140 male albino rats. The rats were divided into 3 groups (control, fresh water, salt water). Mean iron concentration in right and left ventricles and mean difference of iron concentration (RVFe–LVFe) in the fresh and salt water drowning are compared with the control using t-test in SPSS program. **RESULTS**: The results revealed a significant decrease in the mean concentration of iron in right and left ventricles in fresh water drowning in GII a (immediately) and in GII b (after 3 hours) in comparison with control. There was a significant increase in the mean difference of iron concentration between right and left ventricles in (GII a) and (GII b) in comparison with the control. There were no significant changes in mean iron concentration in right or left ventricles in GIII a (salt water immediately) or GIII b (salt water 3h) in comparison with the control. There was a significant decrease in mean difference of iron concentration between right and left ventricles in (GIII a) group in comparison with control. There were no significant changes in mean difference of iron concentration between right and left ventricles in (GIII b) in comparison with the control group. There was a significant decrease in mean iron concentration in right and left ventricles in (GII a) and (GII b) in comparison with (GIII a) and (GIII b) respectively. There was a significant increase in mean difference of iron concentration between right ventricle and left ventricles in (GII a) and (GII b) in comparison with (GIII a) and (GIII b) respectively. In samples taken in (GII c) and (GIII c) samples were difficult to be taken due to advanced putrefaction and postmortem clotting.

INTRODUCTION

Several definitions of drowning are available, one of the most classical definitions is “death by drowning is the result of a hampering of the respiration by obstruction of mouth and nose by a fluid medium (usually water) (***Piette and De lettera,***

2006). Drowning accounts for more than one half million deaths annually worldwide (***De Nicola et al., 1997***). About true drowning there are no substantial controversies. A common pathway is the inhalation of water which can pass the alveolo-capillary membrane and reach the circulation (***Modell, 1993)***. Signs of immersion only demonstrate submersion of the body for a period of time but are not signs of drowning ***(Lorin and Paraire, 2003)***.Cadavers found in water may represent a difficult medicolegal problem, as the diagnosis of the cause of death in such condition is one of the most difficult tasks that can face the medicolegal examiner.

The external signs of drowning (e.g. froth, cadaveric spasm, coolness of the body and goose skin) as well as the internal signs (e.g. the respiratory passages and lung appearance) may be found in other forms of asphyxia or may be difficult to demonstrate especially in massive putrefied bodies ***(Rammer and Gerdin, 1976)***. Most diagnosis is based on a series of nonspecific autopsy finding such as froth around the mouth and nostrils and lung distension. In addition the results of laboratory tests such as the presence of diatoms and biochemical markers ***(Azparren et al., 2000; Zhu et al., 2003A***). In many cases autopsy finding or the presence of diatoms in closed organs merely indicates liquid penetration into the airways during the vital period but not necessarily death by drowning ***(Hadley and Fowler, 2003; Zhu et al., 2003B; Zhu et al. 2003C)***.Many methods have been used to help for this diagnosis, including biochemical methods. Animal experimentations have shown that in typical fresh water drowning `in a hypotonic medium in relation to the blood, haemoglobin and electrolytes such as sodium, chloride and others diminish in the left side of the heart as a consequence of the absorption of water from the lungs into the circulation ***(Swan and Spafford, 1951)***.

De La Grandmaison et al. (2006) found that iron seems to be a good biochemical marker in fresh water drowning with a short postmortem interval. For this reason the present study aimed to measure the postmortem level of iron in both right and left ventricles of the rat's heart in a trial to use it as a laboratory marker for diagnosis of drowning and to differentiate between fresh and salt water drowning.

MATERIALS AND METHODS

Animals

This work was conducted on 140 male albino rats of average weight 100-150 gm each obtained from Assuit University Animal Facility, Faculty of Medicine. They were housed in Animal Facility at Faculty of medicine, Sohag University. All rats were given ad libitum access to Taklad rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes. These conditions were adopted following other groups **(Hussein et al., 2005)**.

Water

Sea water was obtained from the red sea, Horghada. Nile water was obtained from River Nile of Sohag city.

Chemicals:

Kits for determination of iron in blood samples were purchased from Bio-genomics company kit.

Apparatus:

1. JENWAY 6300 spectrophotometer at wave length 535 nm.
2. Centrifuge
3. Vortex homogeneizer (SCIENTIFICA VELP)

Method

After a 7-day acclimatization period, rats were divided into 3 groups:***Group I:*** (20 rats) control group were killed by cervical dislocation.***Group II:*** (60 rats) were drowned in fresh water. This group was subdivided into 3 subgroups each subgroup formed of 20 rats: ***Group (II a*)**: the blood samples were taken immediately**. *Group (II b):*** the blood samples were taken after 3 hours. ***Group (II c):*** the blood samples were taken after 2 days. ***Group III:*** (60 rats) were drowned in salt water. This group was subdivided into 3 subgroups each subgroup formed of 20 rats: ***Group (III a):*** the blood samples were taken immediately. ***Group (III b):*** the blood samples were taken after 3 hours. ***Group (III c):*** the blood samples were taken after 2 days.

Sampling:-

Two blood samples were taken from the heart of each animal by disposable plastic syringe. One was taken from the right ventricle and the second was taken from the left ventricle. *According to the method of* ***De la Grandmaison et al. (2006*)** the following steps were done for all blood samples. Hemolysis is induced by freezing and unfreezing followed by vortex homogeneization.100 µl of blood is diluted with 900 µl distilled water, followed by sample stirring.

Biochemical analysis:-

Using bio-genomics company kit. catalog No. IR 1510, proteins in the blood samples were precipitated out by adding 1 ml from R2 and then let stand for 10 minutes then, the mixture was centrifuged for 10 min. at 3000 rpm, leaving the *Fe* in solution.

***Technique*:**

Biochemical estimation of iron was done on the base of colorimetric technique. When a certain reagent is added to the prepared samples, it forms with iron a complex that can be measured photometrically at certain wave length.

Table (1): Amount of reagent added to the prepared samples

	Blank **ml**	**Standard ml**	**Sample ml**
Supernatant	**-**	**-**	**1**
R1	**-**	**0.5**	**-**
R2	**0.5**	**0.5**	**-**
Dist. Water	**0.5**	**-**	**-**
R3	**1**	**1**	**1**

Using R3 for the colorimeteric determination of iron absorbance in prepared samples was done by adding 1ml of R3 to 1ml from supernatant, the mixture was incubated for 5 min. at 20-25˚c ***(Dreux C., 1977)***.Fe absorbance is measured at wave length 535 nm by spectrophotometer. Before the unknown sample is measured, a blank was made to adjust the instrument to a solution without any iron present. The spectrophotometer was also calibrated with a series of known concentration solutions.*Calculation of iron concentration in the samples:*

$$\textbf{Iron conc.} = \frac{\text{Sample absorbance}}{\textit{Standard absorbance}} \textbf{ X Standard conc. X10}$$

Statistical analysis of results:-

Mean iron concentration in right and left ventricles and mean difference of iron concentration (RVFe–LVFe) in the fresh and salt water drowning are compared with that of control using t-test in SPSS program.

RESULTS:

This work was performed to study the diagnostic value of iron in fresh and salt water drowning, by investigating the postmortem levels of iron in the left and right ventricles of the heart in cases of drowning compared to control cases through experimental study on the rats. ***Table (2) and figure (1)*** show mean iron concentration (µmol/l) in fresh water drowning (GII a, b).

There was a significant decrease in the mean concentration of iron in right and left ventricles of (GII a) in comparison with control ($p<0.001$&0.0001 respectively).There was a significant increase in the mean difference of iron concentration between right and left ventricles of (GII a) in comparison with control ($p<0.0001$).There was a significant decrease in the mean concentration of iron in right and left ventricles of (GII b) in comparison with control ($p<0.001$&0.0001 respectively).There was a significant increase in the mean difference of iron concentration between right and left ventricles in (GII b) in comparison with control ($p<0.0001$).

Table (3) and figure (2) show mean iron concentration (µmol/l) in salt water drowning (GIII a, b).There were no significant changes in mean iron concentration in right or left ventricles of (GIII a) in comparison with control group.There was a significant decrease in mean difference of iron concentration between right and left ventricles of (GIII a) in comparison with control group ($p< 0.0001$).There were no significant changes in mean iron concentration in right or left ventricles or mean difference between right and left ventricles of (GIII b) in comparison with control group.

Table (4) and figure (3) show mean iron concentration in fresh and salt water drowning (GII a, III a).There was a significant decrease in mean iron concentration in right and left ventricles of (GII a) in comparison with (GIII a) ($p< 0.01$&0.0001 respectively).There was a significant increase in mean difference of iron concentration

between right ventricle and left ventricles of (GII a) in comparison with (GIII a) ($p<0.0001$).

Table (5) and figure (4) show mean iron concentration in fresh and salt water drowning (GII b, III b).There was a significant decrease in mean iron concentration in right and left ventricles of (GII b) in comparison with (GIII b) ($p<0.001$&0.0001 respectively).There was a significant increase in mean difference of iron concentration between right and left ventricles of (GII b) in comparison with (GIII b) ($p<0.0001$).

Table (2): Mean iron concentration (µmol/l) in fresh water drowning (GII a, b):

Level / Site	Control		Immediate		P value	3 hours		P value
	mean	S.D.	Mean	S.D.		Mean	S.D.	
Rt	**362.36**	**72.23**	**282.61**	**64.47**	**<0.001*** **	**249.08**	**41.07**	**<0.001**
Lt	**358.36**	**67.59**	**210.64**	**51.63**	**<0.0001****	**174.90**	**30.27**	**<0.0001**
Difference	**4.00**	**9.84**	**71.964**	**59.24**	**<0.0001**	**74.18**	**38.24**	**<0.0001**

* <0.05 significant

** <0.01 mild significant

*** <0.001 moderate significant

**** <0.0001 highly significant

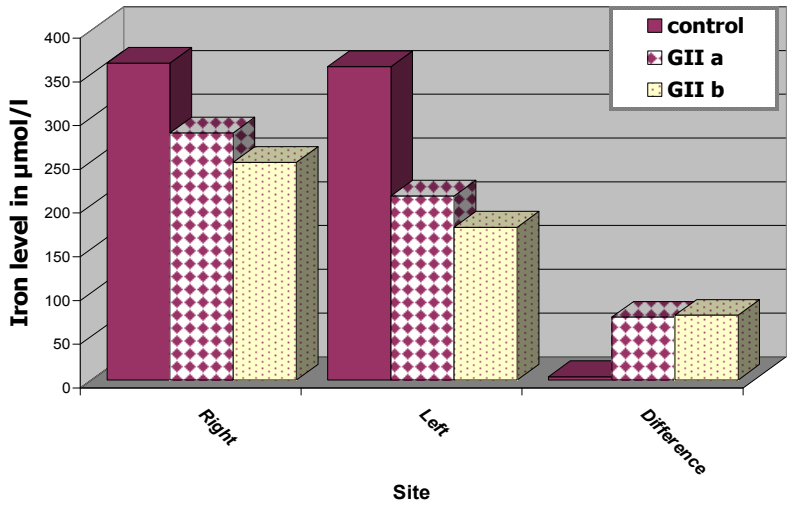

Figure (1): Iron level in fresh water drowning

Table (3): Mean iron concentration (µmol/l) in salt water drowning (GIII a, b):

Level / Site	Control		Immediate		P value	3 hours		P value
	mean	S.D.	Mean	S.D.		Mean	S.D.	
Rt	362.36	72.23	344.04	69.59	>0.05	405.81	63.97	>0.05
Lt	358.36	67.59	354.78	73.09	>0.05	405.78	63.44	>0.05
Difference	4.00	9.84	-10.74	8.56	<0.0001	0.03	7.399	>0.05

* <0.05 significant ** <0.01 mild significant

*** <0.001 moderate significant **** <0.0001 highly significant

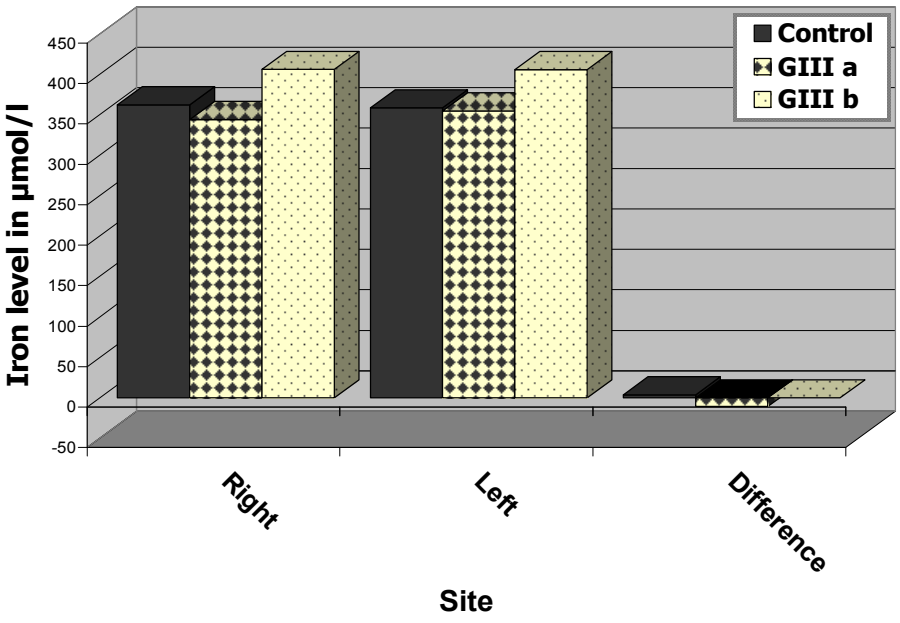

Figure (2): Iron level in salt water drowning

Table (4): Mean iron concentration in fresh and salt water drowning (GII a, III a):

Level / Site	Fresh		Salt		P value
	Mean	S.D.	mean	S.D.	
Rt	282.61	64.47	344.04	69.59	<0.01
Lt	210.64	51.63	354.78	73.09	<0.0001
Difference	71.964	59.24	-10.74	8.56	<0.0001

* <0.05 significant ** <0.01 mild significant

*** <0.001 moderate significant **** <0.0001 highly significant.

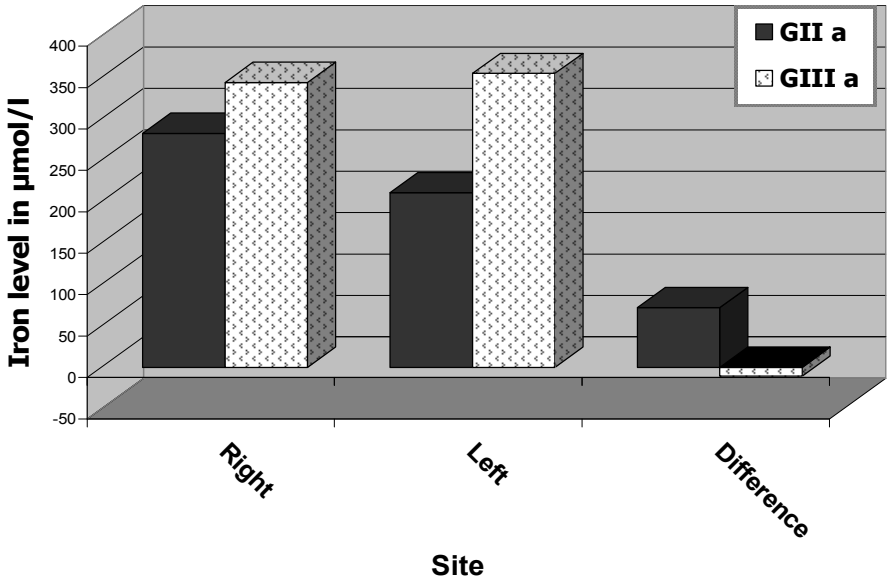

Figure (3): Iron level in fresh and salt water drowning immediately after death

Table (5)*:* Mean iron concentration in fresh and salt water drowning (GII b, III b):

Level / Site	Fresh		Salt		P value
	Mean	S.D.	mean	S.D.	
Rt	249.08	41.07	405.81	63.97	<0.001
Lt	174.90	30.27	405.78	63.44	<0.0001
Difference	74.18	38.24	0.03	7.399	<0.0001

* <0.05 significant ** <0.01 mild significant

*** <0.001 moderate significant **** <0.0001 highly significant

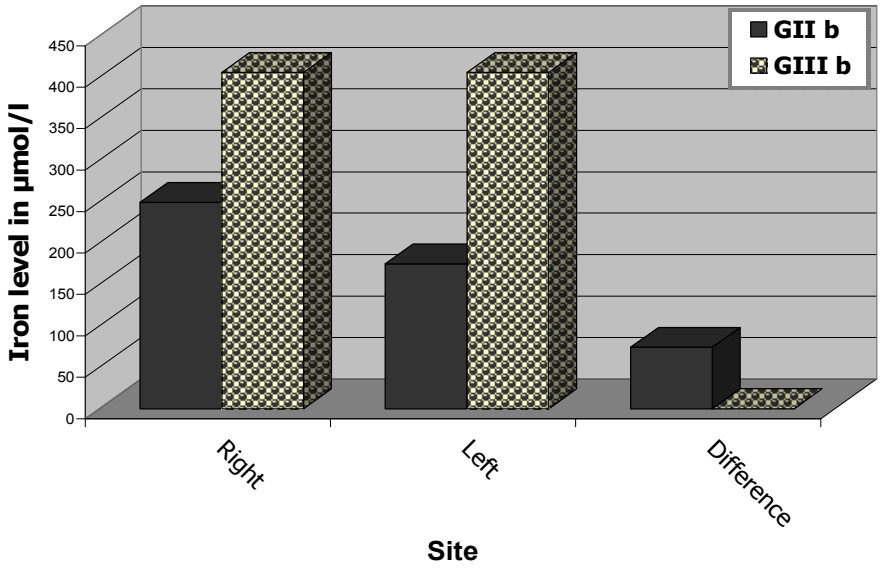

Figure (4): Iron level in fresh and salt water drowning 3 hours after death

DISCUSSION:-

Postmortem diagnosis of drowning is one of the most difficult tasks in forensic pathology. ***De La Grandmaison et al. (2006)*** found that iron seems to be a good biochemical marker in fresh water drowning with a short postmortem interval. For this reason the present study aimed to measure the postmortem level of iron in both right and left ventricles of the rat's heart in a trial to use it as a laboratory marker for diagnosis of drowning and to differentiate between fresh and salt water drowning. This study was done experimentally on the rats to have the advantage of fixing the factors of age, sex, weight and other factors such as feeding, exercise and temperature. The number of animals (140) in the current study was large and included supports reliability of statistical analysis. Rats were found to be suitable animals for this research due to similarity of its body reactions to human being and adequate size of their organs ***(Paine, 1993).***In the present work iron was used as a single parameter for drowning. The present study revealed a significant decrease in the mean iron concentration in right and left ventricles of (GII a) in comparison with control group (GI) ($p<0.001$&0.0001 respectively) *[Table 2& figure 1]*. These results could be explained by the haemodilution which occurred in blood and reached to right and left ventricle. These results were in agreement with ***Conn et al. (1995)*** who found that during cold fresh water drowning, aspiration produced gross hemodilution with an average increase in body weight of 16.5%. Hematocrit values, serum sodium concentrations, and osmolality decreased while free hemoglobin increased. ***Carrara (1902)*** was among the first to apply physical methods to investigate the effects of drowning on the molecular concentration of the blood of the heart by determination of specific gravity, freezing point and electrical conductivity. The author concluded that left ventricular blood is disproportionately diluted after drowning in fresh water and disproportionately concentrated after drowning in sea water .Also the author observed that postmortem

alteration in blood may accounts for the same changes. ***Lougheed et al., (1939)*** found that a comparison of Hb, iron and the number of cells of right and left heart blood constituted a practical method of recognizing death by drowning. It was found that disproportionate dilution of left heart blood occurred after drowning in fresh water, it can be assumed that the nature and degree of changes will depend on the difference that exists between the osmotic pressure of the intra-alveolar fluid and that of capillary blood and on the time that circulation is maintained during and after the inhalation of water. If heart failure in drowning was sudden so as to cause an abrupt and complete cessation of circulation, the finding of significant differences in the composition of blood in the two sides of the heart would be unlikely. ***Modell et al. (2004)*** reported a case of 2 years old boy submerged for at least 20 minute in fresh-water and found rupture in RBCs and release of free plasma haemoglobin and decrease haematocrite value. Contrary to these results ***Modell et al. (1976)*** studied 91 consecutive patients treated for near drowning from 1963 to 1674. The authers stated values for the haemoglobin level and hematocrit reading couldn't be used as indication of type of water aspirated nor the severity of immersion.

The present work showed a significant increase in the mean difference in iron concentration (Rt V Fe-Lt V Fe) between right and left ventricles of (GII a) in comparison with control (GI) ($p< 0.0001$) *[Table 2 & Figure 1]*. These results might be due to the affection of left side of the heart by haemodilution earlier than the right side. These results are in agreement with ***De La Grandmaison et al. (2006)*** who found that a positive comparison between the two sides is helpful for the diagnosis. The mean difference of iron concentration was significantly higher in the drowning cases compared with controls ($P < 0.001$).

The present study showed a significant decrease in the mean iron concentration in the right and left ventricles of (GII b) in comparison with the control (GI) ($p<0.001$&0.0001) *[Table2& Figure 1]*. These results might be due to haemodilution occurred in blood, which reached to right and left ventricles.

On the other hand there was a significant increase in the mean difference of iron concentration (Rt V Fe-Lt V Fe) between right and left ventricle in (GII b) in comparison with control ($p<0.0001$) *[Table2& Figure 1]*. These results occurred because the left side of the heart affected by haemodilution earlier than the right side.

In the current study, there were no significant changes in mean iron concentration in both right and left ventricles of (GIII a) in comparison with control group *[Table3 & Figure 2]*. These results are in agreement with ***Azparren et al. (1998)*** who found that there is no significant difference comparing neither chloride nor Hb biventricular concentrations between typical drowning and atypical drowning cases in blood samples extracted from bodies found in both fresh and seawater. ***Putman et al. (1975)*** found that their study supports the observation of others that distinctive serum biochemical and heamatological values have little relation to the type of fluid medium. Contrary to these results ***Conn et al. (1995)*** found that during cold salt water drowning, average body weight increased by only 6%, with hemoconcentration and shrinkage of vascular volume. Hematocrit and hemoglobin values increased by 30%, but initial plasma free hemoglobin values remained unchanged. Serum sodium concentrations, osmolality, and potassium concentrations increased rapidly to critical levels. Also

Alkan et al. (1977) studied seven cases of submersion in the Dead Sea. They found that haemoconcentration was one of clinical signs found in these cases.

In the present study, there was a significant decrease in mean difference of iron concentration (Rt V Fe-Lt V Fe) between right and left ventricles of (GIII a) in comparison with control group ($p<0.0001$) *[Table 3 & Figure 2]*. These results might be due to affection of the left side of the heart by haemoconcentration earlier than the right side. In this study, there were no significant changes in mean iron concentration in right or left ventricles or mean difference between right and left ventricles of (GIII b) in comparison with control (GI) *[Table 3 & Figure 2]*.

There was a significant decrease in mean iron concentration in right and left ventricle of (GII a) in comparison with (GIII a) ($p<0.01$& $p<0.0001$ respectively) *[Table 4& Figure 3]*. These results could be explained by haemodilution that occurred in fresh water drowning and haemoconcentration that occurred in salt water drowning. Also the difference in agony period between fresh and salt water may contribute another factor explaining the difference in iron concentration between fresh and salt water drowning. These could be explained by the duration of agony. These results were in agreement with ***De La Grandmaison et al. (2006)*** who found a large range of (Rt V Fe– Lt V Fe) levels in the drowning group. The wide variation could be explained by the duration of agony. Also ***Azparren et al. (2000)*** found that intervals of either the difference of strontium concentration between the left and the right ventricular blood or the strontium concentration in the left ventricular blood appear to be related to different time-lapses of the agonal period of drowning.In the present study, there was a significant increase in mean difference of iron concentration between right ventricle and left ventricle of (GII a) in comparison with (GIII a) ($p<0.0001$) *[Table 4 & Figure 3]*. These results could be explained depending on the fact that haemodilution that occurred in fresh water drowning and haemoconcentration that occurred in salt water drowning affected the left side of the heart earlier than the right side.

There was a significant decrease in iron concentration in right and left ventricles of (GII b) in comparison with (GIII b) ($p<0.001$&0.0001 respectively) *[Table 5& Figure 4]*. These results could be explained by haemodilution that occurred in fresh water drowning and haemoconcentration that occurred in salt water drowning.There was a significant increase in mean difference of iron concentration between right ventricle and left ventricles of (GII b) in comparison with (GIII b) ($p<0.0001$) *[Table 5 & Figure 4]*, which could be explained by the previous reason.

In group II c and group III c samples were difficult to be taken due to advanced putrefaction and postmortem clotting. These results were in agreement with ***Jeanmonod et al. (1992)*** who observed that comparison of the blood of the right and left sides of the heart is not useful when the body is altered by putrefaction or when the ventricles are empty or cardiac resuscitation is done which lowers the values and makes the interpretations so difficult.Also ***De La Grandmaison et al. (2006)*** added that iron test was not reliable in advanced putrefaction. It may inferred from the present study that in drowning significant changes of iron content in right and left ventricles of the rat's heart and the difference between them constitutes good evidence in differentiation between fresh and salt water drowning. Unless the blood obtained soon after death, the results are likely to be masked by postmortem diffusion or putrefaction. Moreover, it would be

important to test this marker in human cases to detect its significance and usefulness in drowned cases.

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