Early postmortem biochemical changes and renal immunohistochemical expression of aquaporin-2 to differentiate between saltwater and freshwater drowning: An experimental study

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Abstract: Background:-Examination of immersed bodies is one of the most important aspects in forensic practice. Further differentiation is also an indispensable aspect with respect to determination of freshwater drowning (FWD) or saltwater drowning (SWD). **Objective:-** The present study aimed to investigate the early postmortem cardiac and vitreous biochemical changes and the immunohistochemical expression of aquaporin-2 to differentiate between salt and freshwater drowning. **Methods:-** The study was carried out on 30 adult male Balady rabbits weighted 1.5: 2kg. Rabbits were divided randomly into 3 groups, 10 per each group. Animals received intraperitoneal injection of pentobarbital 50ug/gm. Group(I): served as control, rabbits were sacrificed by cervical dislocation, group (II): FWD model, rabbits administered distilled water (30ml/Kg) at a rate of 1ml/min., and finally group (III): SWD model, rabbits administered (3.5%) NaCl in distilled water (30 ml/Kg) and also in the same rate. Within postmortem interval less than one hour, samples were collected as cardiac blood from right and left ventricles separately, and also the vitreous humor from right and left eyes separately. Serum and fresh incubated vitreous samples were analyzed for levels of biomarkers {K, Na, Cl, Ca, Mg, blood urea nitrogen (BUN), and vitreous urea nitrogen (VUN)}. Kidney specimens were carefully dissected and prepared for immunohistochemical expression of aquaporin-2. **Results:-** The most efficient markers were the high left to right ratio of BUN for hemodilution in FWD model, and the highly elevated left-right ratio of Mg, K, NA, and Cl levels in SWD model. The mixed right and left cardiac serum showed a highly significant elevated K level in FWD, meanwhile in SWD, there was there was a highly significant elevated Na, Cl, and Mg levels with reduced BUN. Vitreous humor showed highly significant elevated Na, Cl, Ca, and Mg levels in SWD and a highly significant elevated K and VUN levels in FWD. Immunohistochemical studies revealed an enhanced expression of aquaporin-2 in the apical plasma membrane of the collecting duct principle cells in SWD group compared with FWD and control groups. **Conclusions:** The current study suggested the usefulness of both serum and vitreous humor biochemical markers, together with the renal aquaporin-2 expression to accurately differentiate FWD from SWD in the early postmortem period. **Aim of the study:-** This study aimed to explore the possible early biochemical and immunohistochemical markers to differentiate between freshwater and saltwater drowning.

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1.Introduction

A body recovered from water does not always imply that death was due to drowning. At present the diagnosis of drowning is routinely based on the combination of a complete autopsy, histopathological findings, toxicological analyses and the diatom test. However, froth around the nostrils and mouth, together with lung distension, pleural effusion, emphysema aquosum, as well as detection of "diatom" test from multiple organs, are supportive but not conclusive evidence of drowning (Piette & Deltter, 2006).

Drowning is still a difficult autopsy diagnosis and it is usually a diagnosis of exclusion. Many proposed biological and chemical markers of drowning are not yet widely accepted. Most of them are based on the physical and biochemical modifications that occur in arterial blood compared to the venous blood due to the marked hemodilution caused by fresh water drowning (inhalation of large volumes of water passing through the alveolar- capillary interface and entering the circulation, or to the hemconcentration with electrolyte shifts in saltwater drowning (Lucci *et al.*,2008).

In forensic pathology, there have been various attempts to use biochemical markers for determining the cause of death. In determining the cause of death, biochemical investigation may be helpful especially in cases of poor morphological evidences e.g., drowning, asphyxiation, poisoning, and acute cardiac death. In

clinical biochemistry, however, serum Ca and Mg are important markers to investigate pathophysiology e.g., in renal, skeletal, muscular, and endocrinal diseases, and also in traumatic skeletal muscle damage involving rhabdomyolysis and seawater near drowning (Zhu *et al.,*2002).

When drowning is diagnosed, it is also indispensable to further distinguish freshwater drowning (FWD) from saltwater drowning (SWD). Macroscopically, the amount of pleural fluid and lung weights show significant differences between FWD and SWD. Changes in FWD are predominantly osmotic. In contrast, abnormal shaped erythrocytes and alveolar epithelium and discontinuity of alveolar lining cells are found in SWD. In addition, several studies reported chemical analyses of serum electrolyte levels including magnesium or immunohistochemical detection of aquaporin-5 expression (Locali *et al.,*2006).

Ancillary studies are pivotal in determining the cause and manner of death in many cases. One of the most ancillary studies is postmortem vitreous chemical analysis, also called vitreous chemistry. Vitreous fluid is ideal for postmortem chemical analysis, as it is relatively isolated from blood and other body fluids that are affected by postmortem changes such as redistribution and hemconcentration. It also resists putrefaction longer than other body fluids and it is relatively protected from degradation and contamination (Collins, 2011).

Bodies found in the sea have not always died from saltwater drowning. At present, the molecular biological techniques have been developed, and widely distributed to the field of medical sciences. Thus, the application of molecular techniques may make forensic diagnosis more objective and corrective (Zubakov *et al.,*2008).

Aquaporins (AQPs) are a family of homologous water channels expressed in many epithelial and endothelial cell types involved in fluid transport, and 13 members (AQP0-13) have so far been identified in mammals. AQPs 1,2 and 4 are the main water channel proteins in the kidney (Hayashi *et al.,*2009).

AQP1 and AQP4 are the main water channel proteins in the brain, which are rapidly induced by various types of stimuli such as osmolarity and chemical or mechanical stress. AQP1 and AQP4 are presumed to be involved in brain neuropsychiatric diseases such as Alzheimer's disease, multiple sclerosis, and schizophrenia. AQP1 and AQP4 appear to be part of mechanism of cerebral volume regulation following ischemia, trauma, tumors, inflammation, and metabolic disturbances. Recently, AQP expression is examined in several human organs from a forensic perspective and it was found that AQPs are suitable as markers for differentiation between FWD and SWD (Hayashi *et al.,*2009).

2.Material &Methods

Animals: A total of 30 adult male Balady rabbits were employed in this experimental work, they were obtained from the faculty of Agriculture-Minia University. Rabbits weighing about 1.5: 2 kg., and they were kept at the constant environmental condition for acclimatization, food and water were provided *ad libitum* for one week before the experiment. Methods: Animals were divided randomly into 3 groups, 10 per each group. They received intraperitoneal injection of pentobarbital 50 ug/gm. Group (I): was the control group, rabbits were sacrificed by cervical dislocation, group (II): was the freshwater drowning (FWD) model, rabbits administered distilled water (30 ml/kg) at a rate of 1ml/min., and group (III): was the saltwater drowning (SWD) model, the rabbits administered (3.5%) NaCl in distilled water (30 ml/kg) and also at the same rate.

Experimental drowning model:-According to (Hayashi *et al.,*2009), a cervical incision (midline) is made, and the trachea is exposed, 26-gauge needle is used for administration of water. Both the terminal gasps and the cardiac arrest are used as the endpoint.

I) Biochemical analyses: The postmortem interval was less than one hour. Blood samples were obtained directly from right and left ventricles separately. Samples were collected aseptically using syringes, then serum was separated immediately by centrifugation and stored at -20ºc until use. Vitreous humor samples were collected as recommended by (Wang *et al.,* 2006), by using a clean sterile syringe with 20-gauge needle separately one for each eye. The needle was inserted through the outer canthus until the tip was placed in the center of the globe and visible through the pupil. Suction was applied gently to withdraw all the humor. Samples were obtained separately from right and left globe. An aliquot of (300ul) of the samples was diluted with 1ml. deionized water and stored at -25ºc. Samples were sent immediately to the Biochemistry Department. Each sample was centrifuged at 3000r.p.m. for 10 minutes and the supernatant fluids were analyzed for the levels of the following biomarkers in cardiac serum and vitreous humor (Na-Ca- Cl -Mg-BUN- and VUN: Bioassay Systems; QuantiChrom Kit). Then, the collected data were subjected to statistics like mean, standard deviation (SD), ANOVA test and Correlation Coefficient (r) test. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16, on Windows XP Professional.

II) Tissue specimens and immunohistochemical studies:- Kidney specimens were carefully dissected and prepared for immunohistochemical examination

by the anti-water channel AQP-2 antibody produced in rabbits (code no. A7310) in a powder form (0.05), and obtained from Sigma Aldrich Company, Egypt. According to (An *et al.,*2009), kidney specimens were examined for expression of AQP2. Kidney segments used for routine histology and immunohistochemistry, were fixed overnight in 10% buffered formalin followed by paraffin embedding. Five-micrometer sections were used for immunohistochemical staining for the polyclonal antibody AQP2 (Côté *et al.,*1993) , briefly, sections were deparaffinized, immersed in 0.3% H2O2 in methanol for 30 min. to eliminate endogenous peroxidase activity, followed by incubation with PBS containing 1% normal serum corresponding to the secondary IgG and 1% bovine serum albumin to reduce nonspecific reactions. The sections were incubated with rabbit anti-human AQP2 (1:1,000) at 4°C overnight. The specimens were then incubated with streptavidin-peroxidase complex (Vector Laboratories) for 5 min at 42°C followed by incubation with 3,3-diaminobenzidine tetrahydrochloride (DAB; Sigma Aldrich, Egypt) for 3 min at 42°C. Slides were counterstained with haematoxylin and mounted. The positive immunoreactivity appeared in the form of brown staining expressed in the apical and basolateral membranes of proximal tubules, and also observed in glomeruli (Bedford *et al.,*2003).

Morphometrical analysis

According to the methods of previous studies (Ishida *et al.,* 2009), morphometrical analysis was performed for semi-quantitative evaluation of immunohistochemical findings. For the evaluation of intrarenal AQP expression, the ratios of the number of AQP2- positive proximal tubules to the total number of corresponding renal tubules were calculated in ten randomly selected fields (×400). The average ratio was evaluated as the AQP expression. Data Handling and Statistics: The means and standard error of the mean (SEM) were calculated for all parameters determined in this study. Statistical significance was evaluated using one-way ANOVA with post hoc testing with the Scheffé's *F* multiple comparisons test or Spearman's correlation coefficient by rank test. $p<0.05$ was considered significant.

3.Results

Biochemical studies:- The average survival time of about 2:3 minutes did not differ among the examined groups. The cardiac serum left to right ratio as shown in table (1), revealed a highly significant elevated BUN level in FWD. The same table showing a highly significant elevated left to right ratio of K, Na, Cl, and Mg levels in SWD. The left to right ratio of Ca level was elevated in SWD but such change was insignificant.

Table(1): Showing left to right ratio of the early postmortem serum cardiac metal concentrations in the three examined groups

	PM. Cardiac serum metal concentrations				
Marker	Control (n=10)	SWD (no=10)	FWD (no=10)	F	*p*
K (mEq/l)	0.81±0.094 0.71:1.00	2.28±0.569 1.50:3.50	1.34±0.169 1.10:1.67	45.83	0.00**
Na (mEq/L)	0.946±0.017 0.92:0.98	2.499±0.030 2.46:2.56	1.38±0.35 1.34:1.43	7875.77	0.00**
Cl (mmol/l)	1.114±0.043 1.00:1.14	2.159±0.087 1.98:2.28	1.34±0.06 1.26:1.42	728.76	0.00**
Ca (mg/dl)	2.592±0.278 2.17:3.00	2.71±1.595 0.46:4.50	2.08±0.198 1.82:2.31	1.28	0.29
Mg (mg/dl)	3.306±1.96 0.43: 4.95	11.33±7.069 4.43:24.25	2.301±0.29 1.83:2.70	13.63	0.00**
BUN (mg/dl)	0.67±0.02 0.64 : 0.7	1.25±0.24 0.5:1.75	3.20±0.32 2.54:3.6	326.3	0.00**

p-value is significant at the 0.05 level

As shown in table (2), when the possible P.M. serum markers of hemdilution in FWD were examined, the cardiac serum K level and BUN were elevated compared with SWD group, such two changes were highly significant. BUN was reduced both in FWD and SWD groups compared with the control group, with the lowest level in SWD group. The early postmortem serum cardiac biomarkers in SWD group showed a highly significant elevated Na, Cl, and Mg levels. ANOVA test revealed insignificant elevated postmortem cardiac serum Ca level in FWD group than SWD group.

As shown in table (3), when the possible vitreous humor markers in SWD were examined, the early postmortem vitreous Na, Cl, Ca, and Mg levels were significantly increased in SWD compared with FWD group. The early P.M.

vitreous urea nitrogen and K levels were elevated in FWD group compared with SWD group, such changes were statistically highly significant.

Table(2): Showing ANOVA test of the early postmortem cardiac serum metal concentrations between the three examined groups

	PM. Cardiac serum metal concentrations				
Marker	Control (n=10)	SWD (no=10)	FWD (no=10)	F	*p*
K (mEq/l)	0.59±0.08 (0.5:0.7)	0.53±0.21 (0.2:0.8)	2.16±0.35 (1.5:2.6)	297.5	0.00**
Na (mEq/L)	17.8±0.54 (16.9:18.5)	113.0±18.52 (90.0:131.7)	70.1±30.83 (39.2:100.45)	109.17	0.00**
Cl (mmol/l)	24.1±1.47 (22.3:25.5)	92.5±14.04 (76.4:110.4)	59.2±22.34 (35.2:83.0)	116.93	0.00**
Ca (mg/dl)	3.49±1.59 (1.5:5.5)	4.96±1.81 (2.8:7.3)	4.38±2.81 (1.0 :9.9)	2.21	0.09
Mg (mg/dl)	0.46±0.38 (0.1:0.99)	1.2±1.09 (0.11:2.9)	0.36±0.15 (0.2:0.55)	42.28	0.00**
BUN (mg/dl)	5.76±1.19 1.0:10.0	2.78±0.47 (4.37:7.08)	3.08±1.65 (2.09:4.09)	17.37	0.00**

p- value is significant at the 0.05 level

Table(3): Showing ANOVA test of the early postmortem vitreous humor metal concentrations between the three examined groups

	PM. Vitreous humor metal concentrations				
Marker	Control (n=20)	SWD (no=20)	FWD (no=20)	F	*p*
K (mEq/l)	0.65±0.01 (0.62:0.67)	0.66±0.25 (0.62:0.69)	0.85±0.13 (0.83:0.88)	775.8	0.00**
Na (mEq/L)	0.29±0.04 (0.251:0.344)	0.89±0.05 (0.827:0.952)	0.13±0.01 (0.121:0.144)	224.0	0.00**
Cl (mmol/l)	0.33±0.04 (0.248:0.374)	0.79±0.10 (0.585:0.897)	0.47±0.08 (0.246:0.571)	180.54	0.00**
Ca (mg/dl)	0.47±0.02 (0.425:0.449)	0.85±0.01 (0.836:0.873)	0.63±0.02 (0.568:0.653)	242.0	0.00**
Mg (mg/dl)	0.18±0.06 (0.1:1.0)	0.9±0.08 (0.8:1.0)	0.32±0.04 (0.3:0.4)	777.5	0.00**
BUN (mg/dl)	0.63±0.01 (0.62:0.65)	0.75±0.01 (0.73:0.78)	1.3±0.17 (1.11:1.87)	250.25	0.00**

p- value is significant at the 0.05 level

For the examined chemical constituents, the correlation between early P.M. cardiac and vitreous chemistry revealed a highly significant positive correlation between Na, Cl, and urea nitrogen levels in FWD, (Table -4).

Table (4): Showing correlation coefficients between P.M. cardiac and vitreous metal concentrations in FWD group

Marker (no=20)	r	*p*
K (mEq/L.)	0.88	0.00**
Na (mEq/L.)	0.98	0.00**
Cl (mmol/L.)	0.88	0.00**
Ca (mg/dl.)	0.01	0.97
Mg (mg/dl.)	-0.32	0.17
UN (mg/dl.)	-0.23	0.34

**Correlation is significant at the 0.01 level (2-tailed)

Table (5) demonstrated a highly significant positive correlation between early P.M. cardiac and vitreous K, Na, and Cl levels in SWD. Also the same table showing insignificant negative correlation between Mg and urea nitrogen levels in cardiac and vitreous chemistry in SWD.

Table (5): Showing correlation coefficients between P.M. cardiac and vitreous metal concentrations in SWD group

Marker (no=20)	r	*p*
K (mEq/L.)	0.883	0.00**
Na (mEq/L.)	0.982	0.00**
Cl (mmol/L.)	0.88	0.00**
Ca (mg/dl.)	0.009	0.97
Mg (mg/dl.)	-0.323	0.165
UN (mg/dl.)	-0.227	0.34

**Correlation is significant at the 0.01 level (2-tailed)

Intrarenal AQP-2 expression was higher in SWD cases. There was a significant increase of intrarenal AQP-2 protein expression in the collecting ducts of the SWD group, compared with the FWD (p=0.00) and control (p=0.00) groups. On the other hand, AQP-2 expression was significantly lower in FWD group than in control group (p=0.00) (Table- 6).

Table(6):-The average ratio and mean ± SD of AQP2-positive collecting tubules in the control and experimental groups.

	The average ratio of AQP-2 expression	X ± SD	*p*
Control group (n=20)	20%	30.2± 1.068	
FWD group (n=20)	10.9%	16±0.447	0.00[c]
SWD group (n=20)	45%	67.4±1.364	0.00[c] 0.00[d]

$p < 0.05$ is significant, [c] versus control group, [d] SWD group versus FWD group

Immunohistochemical studies:

The distribution of AQP2 in kidneys of the control group (Fig.1a) showed that AQP-2 was expressed in the apical plasma membrane and cytoplasm of cells in some collecting ducts.

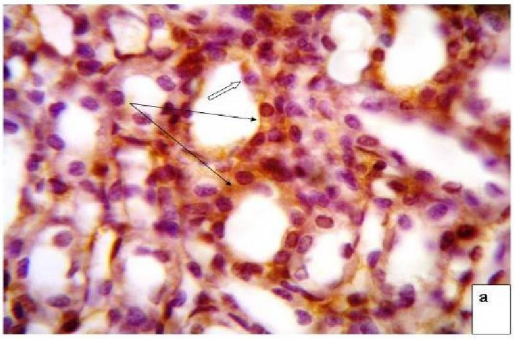

Figure (1-a):- A photomicrograph of the renal medulla immunostained for aquaporin-2 (AQP2) in the control group showing positive cytoplasmic immunoreactivity of principal cells of some collecting tubules (arrows).

In FWD group (Fig.1b), AQP-2 was expressed in the apical plasma membrane and cytoplasm of collecting duct principal cells but appeared to be of lesser extent than in control group.

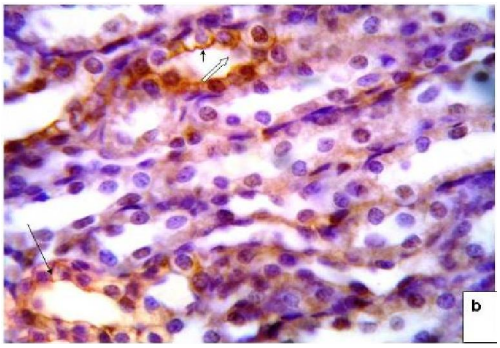

Figure (1-b): A photomicrograph of the renal medulla immunostained for aquaporin-2 (AQP2) in freshwater drowning group showing faint cytoplasmic immunoreactivity of principal cells of the collecting tubules (arrows) and in the apical plasma membrane (arrowhead).

In SWD group (Fig.1c), AQP2 was predominantly expressed at the apical plasma membrane of collecting duct cells and also in their cytoplasm. Moreover, the staining intensity of AQP2 was apparently stronger in the SWD than FWD group. Immunoreactivity was only seen in the principal cells of collecting ducts, with no immunoreactivity of intercalated cells (arrowhead).

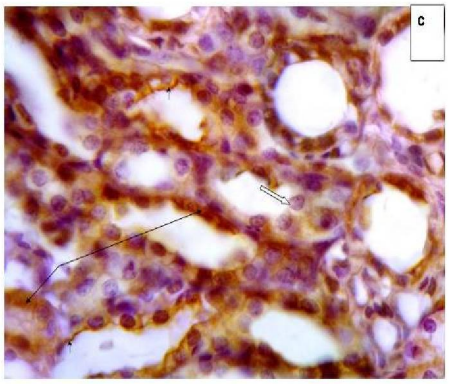

Figure (1-c):- A photomicrograph of the renal medulla immunostained for aquaporin-2 (AQP2) in saltwater drowning group showing extensive cytoplasmic immunoreactivity of cells of the collecting tubules (arrows) and more dense apical plasma membrane immunoreactivity (arrowheads). Notice no labeling of intercalated cells of collecting ducts (white arrow). Paraffin sections, immunohistochemistry, counterstained with H x1000.

4.Discussion:-

Because drowning is due to the inhalation of either fresh or seawater, resulting in lung damage and ventilation-perfusion mismatching, several lines of accumulating evidences have been focused on lung lesions, serum electrolyte concentration, or immunohistochemical detection of intrapulmonary SP-A protein distribution, macrophage amount, and the diatom test. However, there is still no reliable technique for differentiation between FWD and SWD. Recently, several water channel proteins that can regulate osmolarity throughout the body have been cloned (Kwon *et al.,* 2009).

The pathophysiology of drowning depends on various factors including asphyxia and pulmonary damage from aspiration of an immersion medium, a subsequent alteration of blood components, systemic electrolyte and metabolic deterioration. A lack of specific morphological findings may increase the significance of assessment of biochemical evidence (Zhu *et al.,*2002).

Subsequently this study was carried out on an experimental drowning model to demonstrate the early postmortem biochemical changes in the cardiac serum and vitreous humor metal concentrations, together with the immunohistochemical expression of aquaporin-2 in the kidney, to differentiate between freshwater and saltwater drowning. Samples were obtained soon after death within postmortem interval of less than one hour. Unless samples were obtained

soon after death, differences that might otherwise be of diagnostic value are likely to be masked by postmortem diffusion.

Previous studies suggested an early and progressive rise in serum Ca, and Mg levels depending on the time after death (Balbanova *et al.,*1992). In the present study, however, such a postmortem time-dependent rise was avoided by collection and analysis of samples immediately after death.

Non- uniform and unpredictable changes in blood electrolytes which always occur after death render the tests less and less useful the longer the interval between death and recovery of the body (Rammer &Gerdin,1976).

In concordance with (Farmer *et al.,*1985), drowning cases usually showing low serum Na level suggesting aspiration of freshwater. Also, in agreement with the recent results, the same study found a highly significant elevated P.M. serum Cl, and Mg levels which may specially be useful for diagnosis of saltwater drowning.

In agreement with (Farmer *et al.,*1985), The P.M. serum Ca levels was elevated in SWD but such change was statistically insignificant, while the current study does not reveal P.M. increased cardiac Mg levels in FWD, a matter could be referred to the early P.M. collection of the examined samples.

Laboratory methods for the diagnosis of drowning has their rationale in the shift of liquid and electrolytes across the pulmonary air-blood barrier, which may cause blood volume and electrolyte changes. Although, some methods have been reappraised recently, their usefulness is greatly hampered by factors such as the variable volume of drowning liquid penetrating the airways, the differing duration of drowning process, and postmortem biochemical instability (Lunetta *et al.,*2004).

Zhu *et al.*(2005), found that both Ca and Mg levels in the heart and peripheral blood were significantly higher in saltwater drowning compared with those of other groups died from fire fatalities, traumas, myocardial infarction and metamphetamine poisoning. In addition a significant elevation in the Ca level in peripheral blood, was observed in freshwater drowning. Parallel to the current results, analyses suggested a rise in serum Ca and Mg due to aspirated saltwater in seawater drowning.

In another contemporaneous study carried out by (Cárceles *et al.,*2012),the postmortem human biochemical changes revealed a significant elevated Mg, Ca, Na and Cl in left ventricle than right ventricle as a result of water aspiration in saltwater drowning. In contrast, the hemdilution is evident from the significantly higher levels of urea in right ventricle than in left ventricle in freshwater drowning. Such results are coinciding with which was found in this animal study.

The current results are in concordance with (Zhu *et al.,*2003), who showed that the most efficient markers were the left-right cardiac BUN ratio for determination of freshwater drowning (hemdilution) and the left heart blood Mg level for differentiation between fresh- and saltwater aspiration. A characteristic feature of saltwater drowning was a low left-right BUN ratio and a marked elevation in the serum Cl, Mg, and Ca levels of the left heart blood. Saltwater drowning usually showed a characteristic feature of electrolyte and mineral disturbed balance, and is relatively clearly distinguished from freshwater drowning and AMI. Parallel to the recent study, freshwater drowning cases showed a feature of hemdilution and a low serum Na level. Haemdilution in FWD is considered to produce a
lower chloride level in left heart blood when contrasted with right heart blood. Conversely, hemconcentration and chloride ion absorption in SWD is considered to produce the reverse results (Jeanmonod *et al.,*1992).

High levels of plasma magnesium in left heart blood when contrasted with right heart blood is considered to reflect absorption on that ion from the drowning medium particularly saltwater (Karkola&Neittaanmaki,1981).

In a matter concomitant with the explanation of (Philippe&Jerome,2005), in SWD, the hypertonic liquid draws protein-rich liquid from the vascular space into the pulmonary alveoli, causing damage to the basement membrane, dilution and washout of surfactant, and reduction of compliance, pulmonary edema occurs rapidly, and usually within a few minutes the liquid-filled alveoli are incapable of normal gas exchange, which leads to intrapulmonary shunting and a perfusion ventilation mismatch. The shift of liquid into the alveoli results into hypovolaemia and elevated concentrations of serum Na and Cl.

Concurrent to the current results the left- to right ratio of cardiac blood urea nitrogen levels is reduced in SWD and the left cardiac serum Na, Cl, Ca, and Mg levels were higher compared with FWD, meanwhile, the left cardiac serum Ca and Cl levels were lower in freshwater drowning (Maeda *et al.,*2009).

The right-left cardiac ratio of BUN was higher both in SWD and FWD compared with the control group with the most higher ratio level in FWD, in a mater not coinciding with (Zhu *et al.,*2003), who found insignificant difference, which could be referred to analysis of the biomarker levels after a long postmortem interval 48 hours.

Release of potassium during the process of hemolysis in drowning in freshwater stop the heart

suddenly and the hemolysed blood does not then travel through the circulatory system as far as the pulmonary artery. In cases of freshwater drowning in man where the electrolyte values have been estimated within four hours of death, a significant fall may be found in the chloride level in the left side of the heart as compared with the right (Pean,2011). Coinciding with the current results, the mean value of Na levels in vitreous humor was reduced in fresh water drowning, and vice versa it was elevated in saltwater drowning. Alterations in electrolyte levels may have been because of hemconcentrations in SWD or dilution in cases of FWD from electrolyte fluxes in the lungs, or from passive diffusion during immersion (Byard &Summersides,2011).

Given that sodium levels in serum may decrease after death at an average rate of 0.9mEq/L. (Coe,1993), the finding of an elevated sodium level may be of particular significance of SWD (Byramii *et al.,*2008).

In cases where drowning is suspected, the reference values of vitreous humor is preferred as it plays as a mirror of blood. In immersion death there is no intracellular release of Mg and Ca as which happens in fire fatalities, meanwhile, in drowning there is possible post-immersion diffusion of Mg and K across the permeable membranes of the eyeballs, a matter could explain the elevated vitreous Mg and K levels compared to the control group (Farmer *et al.,*1985).

In agreement with the current results, findings of (Byard&Summersides,2011), showing that vitreous analysis for metal concentrations is an easily performed test and can be used to assist in determination of whether immersion was in salt or freshwater. This could be of particular use in a case where a body may have been moved from a bath or waterway after death.

One aspect that is unclear, which is, whether the changes in vitreous electrolyte levels were related to blood electrolyte changes from hemdilution or hemconcentration following inhalation of water, or whether the changes merely reflected diffusion across the external membranes of the eyeball from contact with water during the time of immersion. In SWD, the negative correlation between P.M. Mg, and UN in the cardiac and vitreous chemistry denoting that the vitreous does not usually follow the cardiac changes.

The potential significance of the postmortem vitreous biochemical changes, was studied mainly in multiple literatures for estimation of the postmortem interval. There are scanty literatures concerning postmortem vitreous biochemical changes as a clue for determination of the cause of death.

Changes in the left ventricular blood electrolyte levels in individuals who have been immersed for only short periods of time most likely indicate that electrolyte changes do occur because of ion fluxes across the alveolar membranes (Byard *et al.,*2006).

On the other hand, prolonged soaking in hypotonic or hypertonic solutions might also contribute to these trends in electrolyte levels. Thus, it is possible that at least two mechanisms may have contributed to the electrolyte changes that was found, inhalation and/or diffusion (Rammer & Gerdin,1976).

By ANOVA test the examined vitreous chemical constituents obeying those in the cardiac serum, meaning that: in FWD there were highly elevated K, BUN, and VUN compared with SWD. The same in SWD there were highly significant elevated Cl, Na, Ca and Mg compared with FWD. Although, the Correlation Coefficient test revealed that such changes were not all statistically significant. Aquaporins are small, integral membrane proteins (MW~30.000) that provide a major pathway for water transport in many cell types in fluid-transporting tissues such as kidney, lung and brain, and 13 members (AOP0-12) have so far been identified in mammals (Verkman,2005).

The recent study revealed an enhanced AQP-2 expression in the apical plasma membrane of the collecting duct principal cells in SWD group compared with FWD , and control groups. AQP-2 expression was significantly higher in the control group than in FWD group. Labeling was associated with the apical plasma membrane domains and to intracellular vesicles in apical and basal parts of the cells.

Coinciding with the current work, in An *et al.,*study in (2009), AQP-2 was predominantly expressed in the apical plasma membrane of the collecting duct principle cells in all kidney samples of FWD and SWD. Morphometrically, AQP-2 expression in the apical plasma membrane of collecting ducts was significantly enhanced in SWD group, compared with FWD and control groups.

It is well known that FWD can cause hypervolaemia, marked hemdilution, hemolysis, and the decrease of serum electrolytes, except potassium, by the transportation of hypertonic water into microvessels. The data indicated that AQP2 expression at the apical plasma membrane was down regulated in FWD. Decreased expression of AQP2 at the apical plasma membrane immediately following hypotonic stress may be a protective mechanism limiting apical water entry thus reducing cell swelling and hemdilution in FWD (Tama *et al.,*2007).

Biochemical studies demonstrated that AQP2 could be recycled between the cytoplasm and apical plasma membrane. Once AQP2 is inserted into the apical membrane, water permeability of the collecting ducts increases, enabling water reabsorption. Collectively, it is considered that the apical plasma membrane AQP2-

positive reaction is more bioactive (Nielsen *et al.,*2002).

Conclusion and Recommendations:-

These findings suggested the usefulness of cardiac blood and vitreous humor biochemical markers to differentiate between FWD and SWD in the early postmortem periods. The most efficient markers are the BUN, serum Mg, VUN and the vitreous Na which are helpful to discriminate FWD from SWD.

The current study demonstrated that the reduced vitreous Na level is an easily performed test that may be a useful adjunct to the investigation of possible freshwater immersion death.

The combined biochemical markers together with immunohistochemical studies for marked expression of intrarenal aquaporin-2 is a vital reaction in SWD.

The study revealed the possibility of forensic molecular diagnosis for differentiation between FWD and SWD, and subsequently recommends the combined detection of AQP-2 expression from the renal tissue together with cardiac serum and vitreous biochemical markers in order to more accurately differentiate between FWD and SWD in the early postmortem period.

Efforts should be carried out to obtain blood and vitreous from cases of drowning in humans sufficiently close to death, so that the postmortem changes in electrolyte values can be neglected.

The recent work recommends another comparative study demonstrating blood and other body fluids including pleural and pericardial effusion fluid, following drowning.

Further studies are needed to evaluate whether autolysis have a significant influence on the immunoreactivity of AQP-2.

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