Original Article

Expression of the Heart Fatty Acid-Binding Protein in Heart and Brain Tissues of Electrically Injured Rats for Determination of Post-mortem Interval

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ABSTRACT

Estimation of heart fatty acid-binding protein (FABP3) levels in cardiac tissue may have a value in the identification of myocardial injury as a cause of electrocution death. **The aim of the work:** To investigate for the first time the post mortem *mRNA* expressions of heart fatty acid-binding protein (H-FABP) by qRT-PCR in the heart and brain of electrocuted rats at different post mortem intervals (PMI). **Methods:** Sixty adult male Sprague Dawley rats were allocated into two main equal groups, G1: control (C): exposed to cervical dislocation and G2: exposed to fatal electrocution (EC) (220 V, 50 Hz, 60 sec). Both groups were subdivided into (C0, C4, C8), and (EC0, EC4, EC8) respectively, corresponding to 0, 4, and 8 h PMI in the C and EC groups. **Results:** The H-FABP *mRNA* expression levels were significantly up-regulated in the heart and brain of the EC group (8.59±1.27, 2.25±0.13) compared to the C group (3.35±0.51, 0.96±0.05) respectively. H-FABP expression levels were significantly up-regulated with PMI prolongation either in the heart (3.23±1.04, 5.25±1.40, 7.93±1.98) or in the brain (1.46±0.21, 1.61±0.33, 1.75±0.38) at 0, 4, and 8 h. The interaction between PMI and treatment showed a significant effect on H-FABP *mRNA* expression levels between EC groups in the heart (5.43±0.12, 8.22±0.06, 12.13±0.04) and brain (1.89±0.03, 2.31±0.03, 2.55±0.07) compared with the C groups in the heart (1.04±0.08, 2.28±0.01, 3.73±0.02) and brain (1.04±0.08, 0.90±0.01, 0.95±0.02) respectively at 0, 4, and 8 h. **Conclusion:** The H-FABP *mRNA* expression could be employed as a sensitive detective biomarker for estimation of PMI in cases of electrocution fatalities.

Keywords: Heart fatty acids binding protein, H-FABP, Electrocution, Post mortem interval, *mRNA* expression.

I. INTRODUCTION

Electricity has its own black side since the frequency of electrocution-related fatal and non-fatal injuries increased rapidly as a consequence of its widespread applications (Beyaztas, 2001). Certain body cells known as electrically excitable cells, normally use bioelectric signals, are the most sensitive tissues to electric injury including neurons of the peripheral and central nervous system as well as cardiac cells, diaphragm, and peripheral muscle cells. Therefore, forensic investigations have focused on the effects of the electric shock on the systems that are regulated by electrically excitable cells including, neurons of the peripheral and central nervous system as well as cardiac cells, diaphragm, and peripheral muscle cells (Bertil and Bertil, 2001). Myocardial injury and heart tissue ischemia have been authenticated to be the main effects of electrocution, also ventricular fibrillation is regarded as the foremost cause of death in electrocution-related fatalities (Guntheti et al., 2014).

Death from electrocution could be diagnosed by investigations of the electrical current markings and the circumstances (Dettmeyer et al., 2014). But in some cases of the low-voltage electrical shock, death takes place without clear morphological findings. Such cases are identified as negative autopsy cases which constitute 1-5% of all of the autopsies (Cohle and Sampson, 2001; Saukko and Knight, 2015), which develops a great challenge faces the forensic investigators to detect the cause of death and to estimate the post mortem intervals (PMI). Correct estimation of PMI is considered one of the most significant and complicated duties of forensic investigators (Ferreira and Cunha, 2013).

During the last decade, mRNA profiling has been conducted as a good modern tool that is well-matched with the present forensic experimental research (Hanson et al., 2018) and successful gene expression analysis via qRT-PCR (Koppelkamm et al., 2010) have been conducted using postmortem specimens. Molecular autopsy is defined as genetic studies of extracted DNA or RNA from cadaveric specimens in negative autopsy cases (Ackerman et al., 2001). Thus crucial information about the cause of death or estimations of time since death can be obtained by thanatotranscriptome studies applicable for molecular autopsies (Scott et al., 2020). Several studies have investigated the post-mortem degradation of a variety of gene transcripts by the RNases, however, other studies have reported possible intact RNA isolation even after several days since death (Trotter et al., 2002; Catts et al., 2005;

Heinrich et al., 2007). Expression of apoptotic gene *Bax* was significantly up-regulated in the heart tissue of electrocuted rats after 1hr PMI (Farag and Dhama, 2016). Also, post-mortem heat stress induced a significant fold increase in the expression of inflammation and apoptosis-related genes in mice brain at 1, 3, 6 h PMI (Halawa et al., 2021).

Mammalian fatty acid-binding proteins (FABPs) are cytoplasmic, low molecular weight proteins (14–15 kD), bind to long-chain fatty acids, and carryings them to cell-specific organelles for storage and metabolism of lipids (Furuhashi and Hotamisligil, 2008). Markedly, heart-type fatty acid-binding protein (H-FABP), also known as (FABP3) is abundantly expressed isoform in the cardiac myocytes (Del Collado et al., 2017) and in the brain but in lesser concentrations than those of the heart (Lescuyer et al., 2005). In every area of the brain, the H-FABP concentration was at least 10 times greater than that of brain-type FABP (B-FABP) (Pelsers et al., 2004). H-FABP was discovered to be released by the injured myocardium (Glatz et al., 1988) and was the earliest available marker for cardiac injury (Nakata et al., 2003) owning to its cardiac specificity and rapid releasing during the beginning of ischemic changes (Slot et al., 2010) which makes it a useful marker for ongoing myocardial damage. Furthermore, H-FABP and B-FABP were elevated after electroconvulsive therapy (Pelsers et al., 2004; Özdemir et al., 2016), in ischemic and hemorrhagic stroke (Zimmermann-Ivol et al., 2004), and CO poisoning (Yardan et al., 2011). H-FABP could be used to differentiate the normal myocardial cells from ischemic damaged ones, which is useful for postmortem exploring the cause of sudden death (Hansen and Rossen, 1999) and its ability seemed to be not modified by putrefaction as H-FABP depletion could be detected as long as 60 h postmortem on human hearts (Meng et al., 2006). Recently, the stain depletion of H-FABP reflects the highly significant effect of electric shock on the functional and/or the structural well‑being of human heart tissue which will assist in the diagnosis of heart tissue damage as a cause of death following electrical injury (Kathum and Al-Khateeb, 2019).

Once more, due to the recent refinement in the search for forensic tools especially in the detection of PMI in death and to the best of our knowledge there was no research in the literature until date related to the relative *mRNA* expression levels of H-FABP gene in the heart and brain tissues of male rats at different PMI of electrocution death. Thus, the present study aimed to follow up the H-FABP *mRNA* relative expression as a biomarker for the determination of PMI in heart and brain tissues of electrically injured rats.

II. MATERIAL AND METHODS

II.1 Animal maintenance

A total number of sixty pathogens free adult male Sprague–Dawley rats of the same age (8 weeks old), and the same weight 200±20 g, were provided by Laboratory Animal's Unit of Zagazig Veterinary Medicine (El-Sharkia, Egypt). All rats were left in standard cages with free access to standard pellet feed and water and maintained at room temperature. All procedures applied in our current experiment were approved by the Ethics Committee of Faculty of Veterinary Medicine, Zagazig University, following the guiding principles of The National Institutes of Health Animal Care and Use Committee. All relevant ethical considerations and the animal's euthanasia guidelines were met.

II.2 Animal groups and sampling

Rats were divided into two main equal groups I and II (each of 30 rats). At zero time, the rats from group I: were deeply anesthetized with sodium pentobarbital then were sacrificed by cervical dislocation without any preceding electrical stimulus, and were defined as a control group (C). Rats from group II: were deeply anesthetized with sodium pentobarbital then electrocuted by a 220 V using an electrical energy transfer device that consisted of a double copper cable with a pair of ends. One was peeled 1 cm in length and the other was connected to an electrical energy source (delivering the usual household alternating current of 220 V). The animals were fixed on a plate. The peeled cable is used to provide an electrical current via an anode and a cathode. The anode connected to the left foreleg and the cathode to the right hind leg, rats were electrocuted (220 V, 50 Hz) until death (Wright and Davis, 1980). Rats from this group were defined as the electrocution group (EC). Each rat from both groups was placed in a single plastic sack with pores to allow gases transfer and then kept at room temperature (21–24ºC) for the designated sampling PMI. The intervals of sampling used were: zero time, 4h, and 8h corresponding to C0, C4, and C8 for the (C) group and EC0, EC4, and EC8 for the (EC) group. For each sampling PMI, brain and heart tissue samples (30 mg) were collected from each of 5 rats/ group. The collected samples were frozen directly in liquid nitrogen and stored at -80^{0}C for real-time PCR analysis.

II. 3 Transcriptional analysis of H-FABP in rat heart and brain using quantitative real-time PCR

Total RNA was extracted from the heart and brain tissues (5 samples/ group) using RNeasy mini kit (Qiagen, Cat No: 74104) following the manufacturer's instructions protocol. The purity of RNA samples was tested using NanoDrop spectrophotometer

(NanoDrop technologies, Wilmington, Delaware, USA). The first-strand cDNA was reverse-transcribed from 1µg of total RNA using a Revert Aid Reverse Transcriptase (Thermo Fisher) (200 U/µL). Catalog number: EP0441 by following the manufacturer's instructions. Quantitative RT-PCR was done using SYBR-green detection kit (Applied Bio systems) where 20µl PCR reaction mixture was made by using 150 ng cDNA, 1 µm forward and reverse primer with 10 µl SYBR premix EXtaq and complete the volume using RNase free water. The amplification reaction was carried out using Rotor-Gene Q2 plex (Qiagen Inc., Valencia, CA, USA). The oligonucleotide primers sequence obtained from Metabion (German) for the examined gene H-FABP and the β-actin gene as a house keeping gene to normalize examined gene expression levels. The primers sequences were as follows: H-FABP gene, forward primer: 5-ACGCCTTTGTCGGTACCTGGA- 3 and reverse primer: 5-GGTCATGCTAGCGACCTGTCT-3 (Machida et al., 2014), while the primer sequence for the *β-actin*, forward primer: 5-TCCTCCTGAGCGCAAGTACTCT -3 and the reverse primer: 5-GCTCAGTAACAGTCCGCCTAGAA -3 (Banni et al., 2010). The PCR cycling conditions included an initial denaturation at 95 °C for 12 min followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. The relative fold changes in the expression of the target genes were estimated by the comparative 2−ΔΔCT (Ct: cycle threshold) method with the β-actin gene as an internal control to normalize target genes expression levels (Livak and Schmittgen, 2001).

II.4 Histopathological examinations

The samples of the heart and brain tissues from each group were dissected and were fixed in 10% phosphate-buffered formalin, dehydrated with ethanol, infiltrated, and embedded in paraffin. The layer thickness of the section is 5 µm that routinely examined under a light microscope after staining with hematoxylin and eosin dye (Bancroft and Gamble, 2008). The pathological changes in these tissues were then evaluated by a pathologist.

II.5 Data analysis

All obtained raw data were statistically analyzed using a two-way analysis of variance (ANOVA) followed by post hoc Duncan test to make multiple comparisons test between average of the different groups. A p-value of <0.05 was considered statistically significant. Data were expressed as mean ± SE.

III. RESULTS

Changes in H-FABP transcriptional levels in heart and brain of electrocuted rats

Transcriptional levels of H-FABP in the heart and brain tissues of electrocuted rats compared to the control ones at the different PMI 0, 4 and 8 h are summarized in Table 1. Regarding to the effect of time the obtained results notably showed, a significant ($p < 0.001$) up-regulation of H-FABP *mRNA* expression level either in the heart (3.23±1.04, 5.25±1.40, and 7.93±1.98) or in the brain (1.46±0.21, 1.61±0.33, and 1.75±0.38) respectively with increasing the PMI at 0, 4, and 8 h. Regarding the effect of treatment on the H-FABP *mRNA* expression level, it was showed that the EC group had significantly ($p < 0.001$) up-regulated H-FABP *mRNA* expression levels (8.59±1.27) compared to the corresponding control group (3.35±0.51) in the heart and the EC group had significantly ($p < 0.001$) up-regulated H-FABP *mRNA* expression levels (2.25±0.13) compared to the corresponding control group (0.96±0.05) in the brain

Regarding the effect of interaction between PMI and the exposed group on the H-FABP *mRNA* expression level, it was recorded that the interaction showed a significant ($p < 0.001$) up-regulation of H-FABP *mRNA* expression level between the EC groups in the heart EC0, EC4, EC8 (5.43±0.12, 8.22±0.06; 12.13±0.04) when compared with the corresponding control C0, C4, and C8 groups (1.04±0.08; 2.28±0.01; 3.73±0.02) respectively at 0, 4, and 8 h. Similarly, in the brain, the interaction between PMI and treatment showed a significant ($p < 0.001$) effect on H-FABP *mRNA* expression level between the EC groups EC0, EC4, EC8 (1.89±0.03, 2.31±0.03; 2.55±0.07) when compared with the corresponding control C0, C4, and C8 groups (1.04±0.08; 0.90±0.01; 0.95±0.02) respectively at 0, 4, and 8 h. however, the interaction effect was non-significant between the control groups in the brain at different PMI 0, 4, and 8h.

Table 1. Postmortem relative *mRNA* expression levels of H-FABP in heart and brain tissues of electrocuted rats compared to the control groups at PMI of 0, 4, and 8 h. 5 samples/ group. Using a two-way analysis of variance (ANOVA) followed by post hoc Duncan test.

H-FABP transcriptional expression level		Heart (Mean ± SE)	Brain (Mean ± SE)
Post mortem interval effect			
0 h		3.23±1.04 [c]	1.46±0.21 [c]
4 h		5.25±1.40 [b]	1.61±0.33 [b]
8 h		7.93±1.98 [a]	1.75±0.38 [a]
Significance		***	***
Treatment effect			
Control		3.35±0.51 [b]	0.96±0.05 [b]
Electrocution		8.59±1.27 [a]	2.25±0.13 [a]
Significance		***	***
Interaction effect (Time × Treatment)			
Control	0 h	1.04±0.08 [f]	1.04±0.08 [d]
	4 h	2.28±0.01 [e]	0.90±0.01 [d]
	8 h	3.73±0.02 [d]	0.95±0.02 [d]
Electrocution	0 h	5.43±0.12 [c]	1.89±0.03 [c]
	4 h	8.22±0.06 [b]	2.31±0.03 [b]
	8 h	12.13±0.04 [a]	2.55±0.07 [a]
Significance		***	***

Data are presented as the mean ± SE. SE: standard error. Means within the same column carrying different superscripts are significantly different at ($P < 0.05$). *** = $p < 0.001$.

Histopathology of heart and brain

Lesion severity scoring in the different experimental groups is shown in Table 2. Examined heart sections from the C0 group (Figure. 1.A) revealed aortic wall showed hyalinization of the elastic fibers and connective tissue cells. Hyaline degeneration, vacuolation, and necrosis of the ventricular muscles were seen (Figure. 1.B). Intravascular coagulation, hemorrhage, and hemosiderosis were also seen. Sections from the C4 group (Figure 1.C) showed hyaline degeneration, coagulative necrosis, and autolytic changes in a moderate number of cardiac myocytes could be detected. Intravascular blood coagulation, hemolysis, and hemosiderosis were also observed. Sections of heart from the C8 group (Figure 1.D) revealed intramuscular hemorrhage, hemolysis, hyaline degeneration, necrosis of some cardiac muscle fibers, and autolytic changes in others besides intra and extravascular coagulation of blood were also observed.

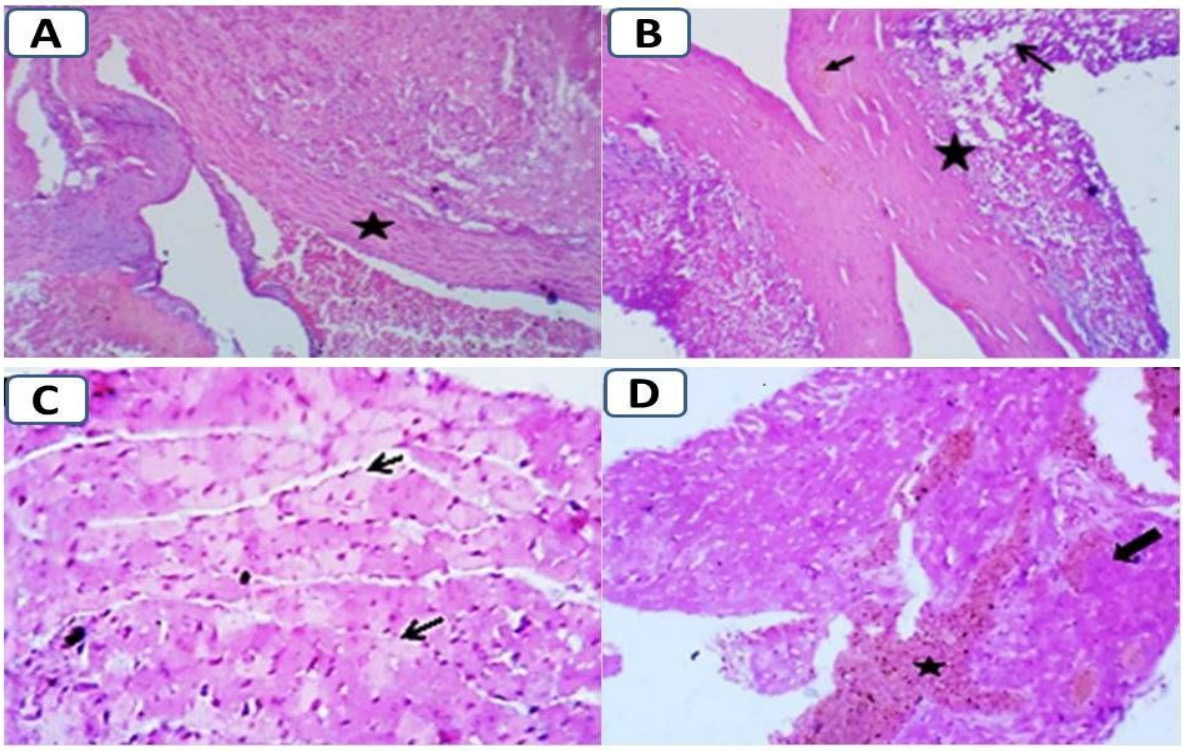

Figure 1: Photomicrograph of the rat's heart sections from the C0 group showing: **A:** Low magnification to show hyalinization of the elastic fibers of the aortic wall (star) X200, **B:** Necrosis of the ventricular muscles (star). **C:** rat's heart sections from the C4 group showing: coagulative necrosis and autolytic changes in a moderate number of cardiac myocytes (arrows) X400. **D:** rat's heart sections from the C8 group showing: intramuscular hemorrhage and hemolysis (star), hyaline degeneration, necrosis, and autolytic changes in some cardiac muscle fibers (arrows) X400.

Examined heart sections from the EC0 group (Figure. 2.A) revealed dilation of coronary and intramuscular blood vessels was seen. Intramuscular hemorrhages together with hyaline degeneration in some cardiac muscle fibers (25-30%) were seen (Figure. 2.B). Sections from the EC4 group showed dilation of the coronary and intermuscular blood vessels with intravascular coagulation and intermuscular hemorrhages were detected (Figure. 2.C). Degenerative changes (cloudy swelling and hyaline degeneration) (Figure. 2.D) in a variable number of cardiomyocytes and focal coagulative necrosis in some muscle fibers were also seen. Sections of the heart from the EC8 group (Figure. 2.E) revealed massive hyaline degeneration and coagulative necrosis of the muscular coat of the aorta and the cardiac muscle fibers were seen. Perivascular edema, dilatation of the coronary, and intermuscular blood vessels, and intravascular blood coagulation were also detected.

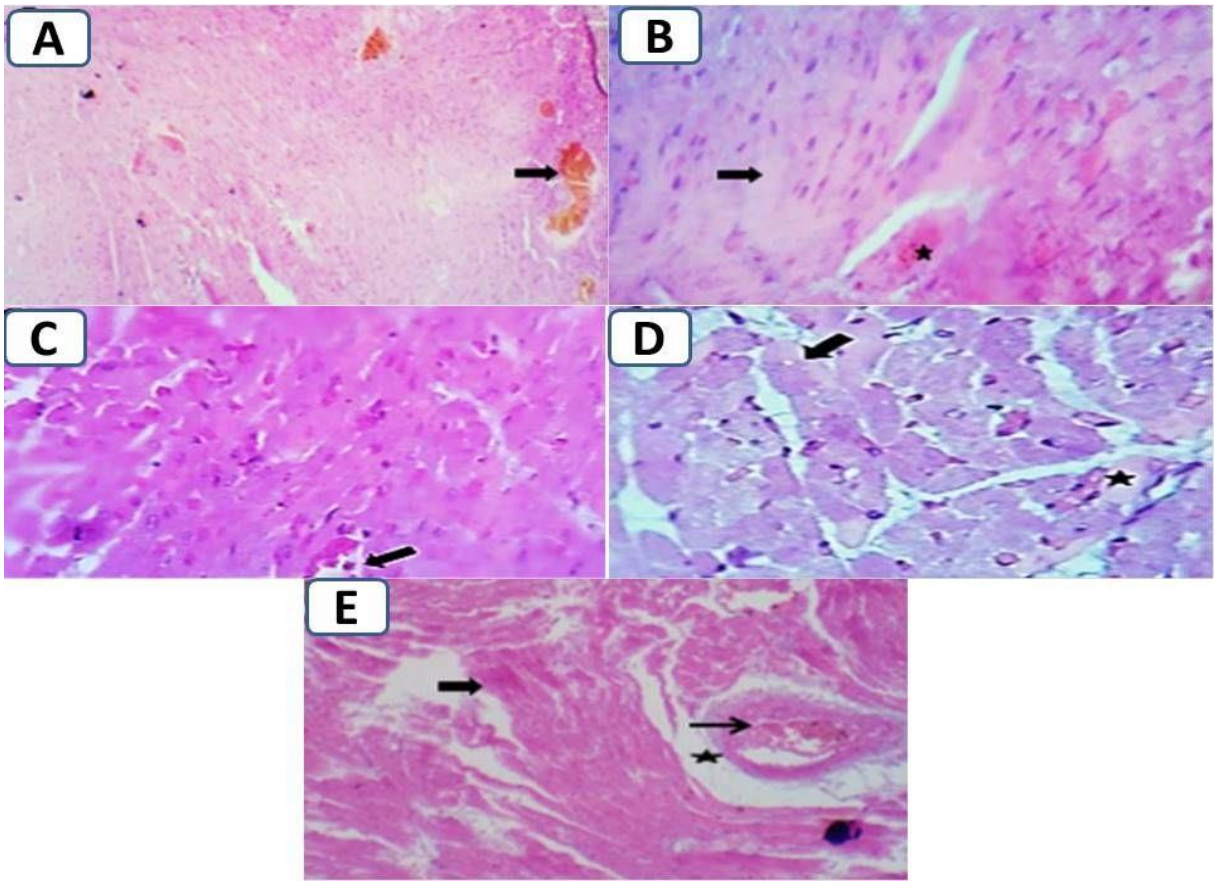

Figure 2: Photomicrograph of the rat's heart sections from the EC0 group showing **A:** Dilation and congestion of coronary and intramuscular blood vessels (arrow), X200, **B:** Hyaline degeneration (star) and necrosis of some cardiac muscle fibers (arrow), X200. Sections from the EC4 group showing **C:** Intramuscular hemorrhage (arrow) X200, **D:** Hyaline degeneration (star) and necrosis of some cardiac muscle fibers (arrow), X400. Heart sections from the EC8 group showing **E:** hyaline degeneration and necrosis of some cardiac muscle fibers (thick arrow), perivascular edema (star) and intravascular blood coagulation (thin arrow), X400.

Examined brain sections of the C0 group (Figure. 3.A) revealed sub-meningeal and cerebral hemorrhage, neuronal degeneration in about 25% of the cells besides satellitosis, and neuronphagia. Sub-meningeal demyelination of some nerve fibers (Figure. 3.B) and intravascular blood coagulation were also observed. While the examined brain sections of the C4 group (Figure. 3.C) revealed massive neuronal degeneration, satellitosis, and neuronphagia in 60-70%of cells, focal vacuolation and demyelination of the nerve fibers (Figure. 3.D), intravascular blood coagulation, sub-meningeal and cerebral hemorrhage with coagulation and hemolysis of erythrocytes. Examined brain sections of the C8 group revealed about 70-75% of the cells with a massive neuronal degeneration, satellitosis, and neuronphagia (Figure. 3.E). Axonal demyelination, degeneration of neurons (Figure. 3.F), perivascular hemorrhage, blood coagulation, and hemolysis were outstanding.

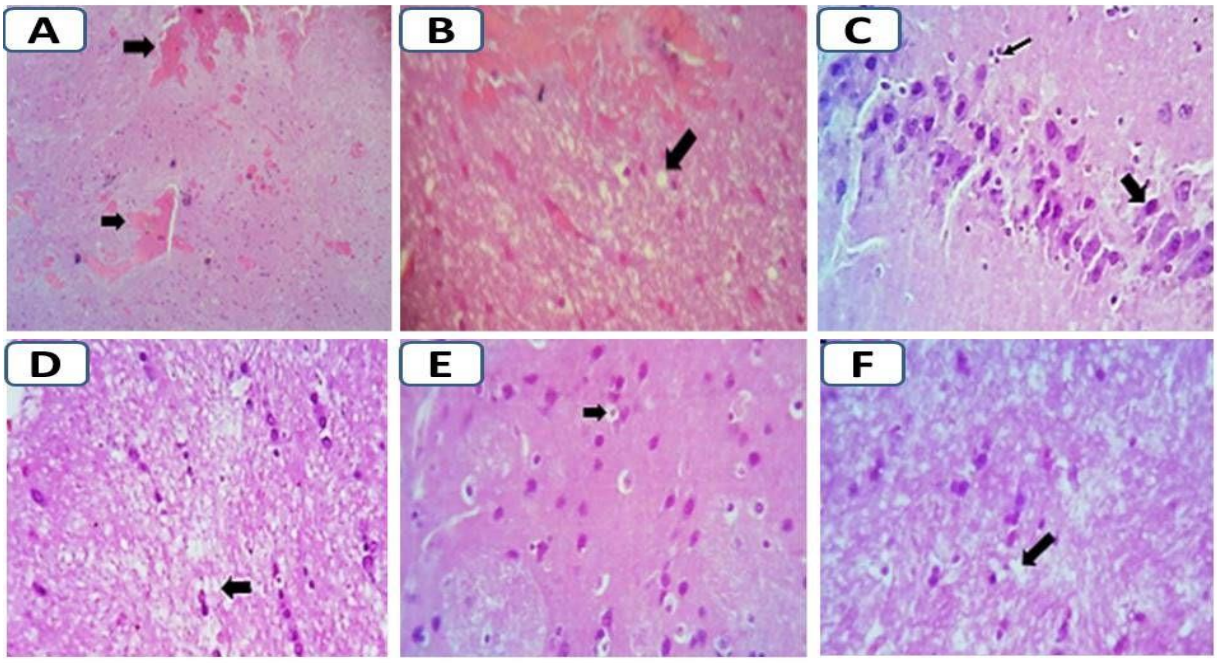

Figure 3: Photomicrograph of the rat's brain sections from the C0 group showing: **A:** cerebral hemorrhage (arrows) X200. **B:** Focal vacuolation and demyelination of the nerve fibers (arrow) X200. Brain sections from the C4 group showing: **C:** Massive neuronal degeneration, satellitosis and neuronphagia (arrows) X400. **D:** Focal vacuolation and demyelination of the nerve fibers X200. Brain sections from the C8 group showing: **E:** Massive neuronal degeneration and neuronphagia (arrow) X400. **F:** Axonal demyelination and vacuolation (arrow) X400.

Examined brain sections from the EC0 group (Figure. 4.A) revealed sub-meningeal, and cerebral hemorrhages, axonal and neuronal degeneration in about 15-25 % of neurons, and intravascular blood coagulation were seen. While examined brain sections of the EC4 group revealed neuronal degeneration of a moderate number of neurons (45-50%) satellitosis, and neuronphagia (Figure. 4.B), intravascular blood coagulation, and Focal axonal degeneration, and demyelination (Figure. 4.C). Examined brain sections of the EC8 group revealed massive neuronal and axonal degeneration in about 65-75% of the cells (Figure. 4.D), focal necrosis of the granular cell layer of the cerebellum and some of the Purkinje cells were seen (Figure.4.E), Dilatation of the meningeal and cerebellar blood vessels with intravascular coagulation was seen. Focal hemorrhages, perineural edema and demyelination of some neurons were also detected (Figure. 4.F).

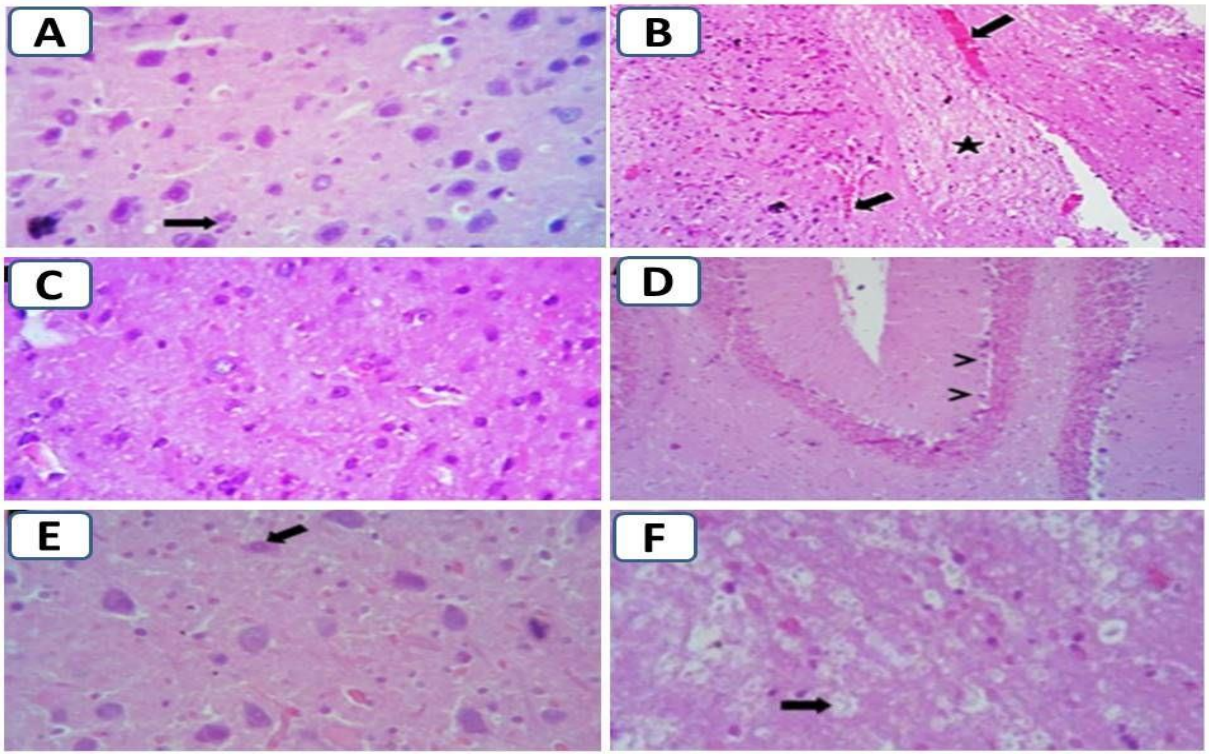

Figure 4: Photomicrograph of the rat's brain sections from the **EC0** group showing **A:** neuronal degeneration and neuronphagia (arrow) X400. Brain sections from the **EC4** group showing **B:** diffuse demyelination (star) and hemorrhages (arrows) X200. **C:** Massive neuronal degeneration and neuronphagia. X400. Brain sections from the **EC8** group showing **D:** Massive necrosis of the granular cell layer of the cerebellum (arrowheads) X200, **E:** Neuronal degeneration (arrow) and hyperplastic microglia beside dilatation of the capillaries X400, **F:** Axonal demyelination and vacuolations (arrow). X400.

Table 2: Lesion scoring in the different experimental group

Criteria	C0	C4	C8	E0	E4	E8
Sub-meningeal and cerebral hemorrhages	+++	++	+	+	+	+
Neuronal and axonal degeneration	++	++++	++++	++	+++	++++
Neuronal necrosis	-	-	-	-	-	++
Peri-neural edema	-	-	-	-	-	+++
Axonal demyelination	+	++	-	-	+	+++
Satellitosis	+	++	+	-	+	-
Myocardial necrosis	+++	++	++	-	+	-
Myocardial hyaline degeneration	+	+	+	++	++	++
Intramuscular hemorrhages	-	-	++	-	-	-
Perivascular Edema	-	-	-	-	-	++

(-=No lesion, += mild, ++= Moderate, +++= severe, ++++= Diffuse)

IV. DISCUSSION

The present study designed to investigate the *mRNA* expression levels of H-FABP gene in the heart and brain tissues as a valuable biomarker for the detection of early PMI after fatal electrocutions in male rats. From the obtained results and to the best of our knowledge, this is the first study showed that H-FABP *mRNA* expression levels significantly upregulated in the heart and brain tissues of electrocuted rats than the corresponding cervical dislocation (control) groups. Additionally, with the prolongation of PMI in the heart and brain tissues, FABP expression was significantly up regulated in all groups except for that of the brain of the control group where the effect of interaction between the PMI and exposed group, induced non-significant changes in the H-FABP expression with prolongation of PMI.

In forensic science, the investigation of gene expression in cadaveric specimens is extensively applied as the changes in the regulation of genes transcriptional levels is critical in relation to various conditions like those happen after death. The postmortem changes result in the release of cellular components including proteins and RNA into tissue fluids. At that time, a diversity of gene transcripts are liable to post-mortem degeneration by the RNases, some studies have reported possible extraction of intact RNA even several days after death (Inoue et al., 2002; Catts et al., 2005; Heinrich et al., 2007). After death cells are expected to start the expression of genes that regulate events related to death. The tissues specific molecular profiles may defend specific mRNAs in one tissue however not the other, or while one manner of death is relevant but not the other, thus shielding the bound mRNA from degradation (Bernstein and Ross, 1989; Ross, 1995). This defense results in a variable half-life for specific mRNA degradation cascade similarly, which may be used to determine the different manners of death. Postmortem mRNA molecules are relatively unsteady; but, the half-lives of mRNAs differ from minutes to days depend on the organ (Vennemann and Koppelkamm, 2010). Postmortem tissues may employed as a practical tool for gene expression investigations based on RNA from autopsy-obtained cardiac tissue during 24 hours of autolysis suggesting that autolysis has relatively minor effects on RNA integrity which revealed biologically relevant expression pattern differences (Gupta et al., 2012).

The postmortem gene expression (thanatotranscriptome) patterns have been utilized to determine the cause and time of death (Scott et al., 2020). Few studies have reported that after death definite genes are still expressed consequently, could be used

as markers for detection of PMI (Franz et al., 2005; Javan et al., 2015). Additionally, post-mortem mRNA expressions of about 1063 genes were up-regulated in the first hour after death, and up to 96 hours in mice brain, then gradually reduced (Pozhitkov et al., 2017). On the other hand, expressions of the same genes were decreased with PMI in the liver tissues.

H-FABP protein content has been reported to become modified in parallel to its *mRNA* level, indicating that modulation of FABP expression is mediated at the transcriptional level (Watanabe et al., 1993). The obtained findings are in agreements with that study reported that in the early PMI of the electrocution, serum levels of H-FABP increased immediately following electrocution suggesting that serum H-FABP levels may be a good biomarker for myocardial injury (Özdemir et al., 2016). Also our results are in harmony with the study in which expression levels of inflammation and apoptosis-related genes were significantly increased in brain of mice exposed to post-mortem heat stress at 1, 3, 6 h PMI (Halawa et al., 2021). Apoptosis is a gene-regulated event and controlled by *Bcl2* family of cytoplasmic proteins including *Bcl-2* and Bax proteins where *Bcl-2* protects cells by inhibiting apoptosis, while Bax enhances apoptosis (Kroemer, 1997; Ashkenazi and Dixit, 1998). As well, genes that encode apoptosis were analyzed in human hepatic tissues from 6 to 48 h after death where, the *mRNA* expressions of pro-apoptotic genes were up-regulated and expressions of anti-apoptotic genes were down-regulated (Javan et al., 2015). Similarly, apoptosis related gene mRNA expression was altered after electrocution death in male rats as reported where, electrocution significantly down-regulate the *Bcl-2* and significantly up-regulate the *Bax* expression in heart of the electrocuted rats (Farag and Dhama, 2016).

Death leads to several sequential events after a person dies. For example, the heart stops beating and the lack of oxygen causes hypoxia within the cells (Di Nunno et al., 1999). Hypoxia and ischemia trigger several factors that lead to the degradation of proteins via proteolytic and autolytic enzymes (Magni et al., 2013). The detection of H-FABP after ischemic lesions of myocardium seemed not affected by putrefaction, as the depletion of H-FABP could be distinguished from normal staining on human hearts as long as 60 h after death. The obtained findings may be attributed to the ischemic or hypoxic conditions which could up regulate the H-FABP expressions as reported in a study which investigated H-FABP expression up-regulation either in vivo via induction of myocytes apoptosis in the infarction and border areas and aggravated cardiac

dysfunction or in vitro promoted drastically death and apoptosis of neonatal rat ventricular cardio-myocytes (NRVM) under hypoxia with lower left ventricular ejection fraction (LVEF) (Zhuang et al., 2019). The study also showed that HIF-1α would activate H-FABP transcription and up regulate its protein level under hypoxia, consistent with the metabolism changes observed after oxygen deprivation.

In fact, tissue-specific FA management can be controlled directly by FABPs themselves via translocation to the nucleus, allowing peroxisome proliferator-activated receptor (PPAR) to bind to the *FABP* promoter and induce transcription (Venkatachalam et al., 2013). Long chain fatty acids (LCFAs) induce both translation and transcription, probably through interactions with peroxisome proliferator activated receptors (PPARs) and concomitant interaction with promoter regions of FABPC genes (Stewart and CMLS, 2000). Thus, the metabolism of lipids appears to be involved in an elaborate feedback system. There are also indications that aging alters the expression of fatty-acid-binding proteins in the mouse brain (Pu et al., 1999).

Overexpression of H-FABP has induced mitochondrion impairment in P19 cells during differentiation which characterized by lower ATP synthesis and lower mitochondrial membrane potential MMP, increased ROS levels, and abnormal mitochondrion morphology. Finally, concerning the correlation between apoptosis and mitochondrion impairment, deformed mitochondria and elevated ROS induced by overexpression of H-FABP may likely be contributors to cell apoptosis (Song et al., 2012).

Electrocution can cause heart tissue damage either directly due to the electro-thermal effect on myocardial tissue represented by coagulative necrosis through the vertical (head to foot) electric current flow or indirectly through ischemic injury precipitated either by arrhythmia-induced hypotension which is common with low-voltage alternating current shock or rarely due to acute coronary artery occlusion (Czuczman et al., 2009). In the present case, necrosis of the myocardial fibers is not accompanied by an inflammatory reaction, which is suggestive of the fact that the death was sudden and there was no time for inflammatory reaction to develop. It appears that the necrotic changes and the marked hyaline degeneration in the myocardium could be used for identification of the electrocution as a cause of death. Electrocution may disturb the heart either by triggering cardiac dysrhythmias or direct myocardium necrosis (Koumbourlis, 2002). In electrocution fatalities, the myocardial injuries were reported by (Ross et al., 1987;

Fish, 1993). Patterns of myocardial morphologic changes ranged from small foci in one area to a diffuse involvement of most or all cardiac areas (Lichtenberg et al., 1993).

V. CONCLUSION

Since electrocution is a probable cause of negative autopsy; therefore, the findings of the current study revealed that H-FABP *mRNA* expression levels are positively up-regulated with the prolongation of PMI in the heart and brain tissues of electrocuted rats, therefore it could be used as a valuable biomarker for detecting of PMI in the electrocution fatal deaths during the early PMI even in the negative autopsy cases of electrocution. Additional, thanato-transcriptome studies are required with different types of genes and types of death.

VI. DECLARATION OF INTEREST

The authors declare that they have no conflict of interest. This research did not receive any specific financials from funding agencies in the public, commercial, or not-for-profit sectors to influence the work reported in the current paper.

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التعبير الجيني للبروتين المرتبط بالأحماض الدهنية للقلب في أنسجة القلب والدماغ للفئران المصابة كهربائيًا لتحديد فترة ما بعد الوفاة

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[1] قسم الطب الشرعي و السموم- كلية الطب البيطري- جامعة الزقازيق

ان قياس مستويات البروتين المرتبط بالأحماض الدهنية للقلب (H-FABP) في أنسجة القلب يستخدم في تحديد إصابة عضلة القلب كسبب للوفاة بالصعق الكهربائي. لذلك كان هدف الدراسة الحالية ولأول مرة قياس التعبير الجيني للبروتين المرتبط بالأحماض الدهنية للقلب (H-FABP) بواسطة qRT-PCR في انسجة القلب والدماغ في الفئران التي تم صعقها بالكهرباء في فترات مختلفة بعد الوفاة (PMI). تم تقسيم عدد ستين من ذكور الفئران البالغة إلى مجموعتين رئيسيتين (كلا منها 30)، المجموعة الضابطة (C): تعرضت لخلع العنق و المجموعة الثانية (EC): تعرضت للصعق الكهربائي المميت (220 فولت ، 50 هرتز ، 60 ثانية) ، والتي تم بعد ذلك تقسيمهما إلى (C0 ، C4 ، C8) و (EC0 ، EC4 ، EC8) على التوالي ، بما يتوافق مع 0 ، 4 ، 8 ساعات من الفترات المختلفة بعد الوفاة (PMI). تم ملاحظة حدوث زيادة في مستويات التعبير الجيني H-FABP بشكل معنوي في انسجة القلب والدماغ لمجموعة الصعق الكهربي (EC) (8.59±1.27 ، 2.25±0.13) مقارنة بالمجموعة الضابطة (C) (3.35±0.51 ، 0.96±0.05) على التوالي. وكذلك زيادة مستويات التعبير H-FABP بشكل معنوي مع إطالة الفترات المختلفة بعد الوفاة (PMI) إما في القلب (3.23±1.04 ، 5.25±1.40 ، 7.93±1.98) أو في الدماغ (1.46±0.21 ، 1.61±0.33 ، 1.75±0.38) عند 0 ، 4 ، 8 ساعات. أظهر التفاعل بين الفترات المختلفة بعد الوفاة (PMI) والصعق الكهربائي تأثيرًا كبيرًا على مستويات التعبير H-FABP بين مجموعات EC في القلب (5.43±0.12 ، 8.22±0.06 ، 12.13±0.04) وفي الدماغ (1.89±0.03 ، 2.31±0.03 ، 2.55±0.07) مقارنة بالمجموعات الضابطة C في القلب (1.04±0.08 ، 0.90±0.01 ، 0.95±0.02) والدماغ (1.04±0.08 ، 0.90±0.01 ، 0.95±0.02) على التوالي عند 0 ، 4 ، 8 ساعات. تشير النتائج التي توصلنا إليها إلى أنه يمكن استخدام تعبير H-FABP mRNA كمؤشر حيوي حساس لتقدير فترة ما بعد الوفاة في حالات وفيات الصعق الكهربائي.