CARDIAC HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICHAL CHANGES DUE TO ELECTRICAL INJURY IN RATS

BY

Nagwa M. Ghandour, Abeer E. Refaiy* and Ghada A. Omran

Departments of Forensic Medicine and Clinical Toxicology; and Pathology,*

Faculty of Medicine, Assiut University

ABSTRACT

The aim of this study was to detect the histopathological findings in the myocardium after electrical injury in rats and the potential role of c- fos expression as a marker to distinguish between antemortem and postmortem electrocution. Seventy healthy female rats were included in the study and randomly divided into four groups (A,B,C,D). Group (A): Twenty rats were subjected to instantaneous antemortem electricity, and were divided randomly into two subgroups. Group (A1): Ten rats were subjected to cervical dislocation and the hearts were collected immediately. Group (A2): Ten rats were left alive for1h from electrical injury then hearts were collected after cervical dislocation. Group (B): Twenty rats were electrically injured instantaneously postmortem, after death by cervical dislocation, and were also divided into two subgroups. Group (B1): Hearts were collected immediately in 10 rats. Group (B2): Hearts were collected after 1h from electrical injury in the other 10 rats. Group (C): Twenty rats were electrified up to death, and divided randomly into two subgroups. Group (C1): Hearts of 10 rats were collected immediately. Group (C2): Hearts of the other 10 rats were collected after 1h of death from electricity. Lastly, 10 rats were served as the control group (Group D). Group (D1): Five rats were clamped but not electrified, before death by cervical dislocation. Group (D2): other 5 rats were clamped but not electrified, after being killed by cervical dislocation. Sections from the hearts of all groups were fixed in formalin and routinely processed. c- fos expression was evaluated in all groups by immunohistochemistry. Significant histopathological findings were detected in groups A and C. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups (A1) and (B1). Positive expression of c-fos protein increased in rats of groups C1, C2 and A2. No c-fos oncogene protein expression was seen either in control groups or in group B2. Significant differences in c-fos oncogene protein expression were observed between rats of groups A1, A2, C1 and C2. Thus, c-fos can be regarded as a marker in identifying electrical injury, and can be used as an indicator to distinguish between antemortem and postmortem electrocution.

Key words: *electrical injury, cardiac, histopathological, immunohistochemistry, C-fos expression.*

INTRODUCTION

It is a challenging forensic task to determine the cause of death in an electrocuted victim without detectable current marks on the skin (Wang et al., 2008 a). In order

to find an effective way for diagnosis of these cases, forensic pathologists have been making lot of efforts to resolve this problem (Wang et al., 2009).

It is a well known fact that electricity can cause death or any degree of damage to various organs and systems according to the type, voltage and intensity of the electrical current and the location of damage. The electrical shock may strike the victim's central nervous system, the cardiovascular system, the skeletal muscular tissue, the lungs, the skin and other internal organs (Fineschi et al., 2006 a).

Cardiac arrest can be induced by a number of mechanisms with little or no tissue damage (Fish, 1993). The principal cause of death was described by Michiue et al. (2009) as cardiac failure due to ventricular fibrillation caused by a direct effect of the electric current.

In forensic pathology, while classical morphology remains a core procedure to investigate deaths, a spectrum of ancillary procedures has been developed and incorporated to detail the pathology (Maeda et al., 2010).

C-fos, one of a small group of genes called primary response genes and its protein product, Fos, are integral components of complex signaling mechanisms believed to be responsible for cell response to stimulation. The effects of many types of stimulation including drug-induced seizures, activation of receptors, growth factors, neuroactive drugs, electrical stimulation, and physiological states have been studied (Krukoff et al., 1992).

The expression of c-fos is known to be increased in particular disorders and pathophysiological processes, indicating that it may play a role in the pathogenesis of some diseases. In rat models of myocardial stunning (MS), the expression of Fos protein increased apparently, i.e. c-fos plays an important role in myocardial lesion, and has close relation to injury repair of the molecule (Zhang et al., 2010).

The aim of this study is to evaluate the effect of fatal and non-fatal electric injury in rats, to characterize the pattern of the structural myocardial changes after electrocution, to study the immunohistochemical expression of c-fos in the heart and to evaluate if it could be used as an indicator to distinguish between antemortem and postmortem electrical injuries.

MATERIALS AND METHODS

Animal groups and Experimental design:

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care followed within the Faculty of

Medicine, Assiut University, as stated by the Institutional Animal Care and Use Committee Guidebook (2002).

Seventy healthy female rats (with average weight 200 ± 50 g) were randomly divided into four groups. The experimental groups were dealt with as follows: rats subjected to antemortem electricity (group A), rats subjected to postmortem electricity (group B), the third group was exposed to electricity up to death (group C), lastly the control group (group D). The rats were subjected to electric current according to the method described by Wang et al. (2006). Two metal clamps were connected to a pole of 220V alternating current. One clamp was connected to rats left hind limbs and other to right forelimbs.

Group (A): Twenty rats were subjected to instantaneous (for 5 seconds) antemortem electricity. This group was divided randomly into two subgroups. Group (A1): Ten rats were subjected to cervical dislocation and the hearts were collected immediately. Group (A2): Ten rats were left alive for 1h from electrical injury and then subjected to cervical dislocation and hearts were collected.

Group (B): Twenty rats were electrically injured instantaneously (for 5 seconds) postmortem, after death by cervical dislocation. This group was divided randomly into two subgroups. Group (B1): Hearts were collected immediately from 10 rats. Group (B2): Hearts were collected 1h after electrical injury in the other 10 rats.

Group (C): Twenty rats were electrified up to death, also divided randomly into two subgroups; each subgroup consisted of 10 rats. Group (C1): Hearts were collected immediately. Group (C2): Hearts were collected after 1h from death due to electrocution.

Group D (the control group): Ten rats were divided randomly into two subgroups; each subgroup consisted of 5 rats. Group (D1): Five rats were clamped but not electrified, for 10 seconds, before death by cervical dislocation. Group (D2): were clamped but not electrified, for 10 seconds, after being killed by cervical dislocation.

Histopathological and immunohistochemical examination:

Sections from collected hearts were fixed in formalin and routinely processed. Five μm sections were cut and stained with hematoxylin-eosin (H & E). The tissue sections were observed under light microscope (Olympus, Tokyo, Japan) for detection of histopathological changes.

Immunohistochemistry (IHC) was performed according to manufacturer's protocol and as previously described by

Zhang et al. (2010). Tissue sections (4-µm thickness) of formalin-fixed, paraffin-embedded specimens were deparaffinized, rehydrated, and transferred to phosphate buffered saline (PBS; PH 7.6). The slides were rinsed twice with PBS, and then endogenous peroxidase was blocked by the hydrogen peroxide for 5 minutes.

Antigen retrieval was done by boiling the slides in citrate buffer (PH 6) for 12 minutes. Then, the slides were washed three times with PBS. The slides were then incubated overnight with primary antibody for c-fos rabbit polyclonal antibody (Cat No. E4460 Spring Bioscience Ca USA) at a dilution of 1:50. The slides were then rinsed three times with PBS and incubated for 10 minutes at room temperature with the biotinylated goat antipolyvalent antibody (Thermo Scientific, Fremont, USA). The slides were rinsed with PBS for three times and incubated for 10 min. with streptavidin peroxidase (Thermo Scientific, Fremont, USA) at room temperature. The slides were then washed with PBS, and diaminibenzidine was applied for 5 min. The slides were then rinsed in distilled water (DW), counterstained with Mayer's hematoxylin, dehydrated and then mounted. Positive control for c-fos antibody was taken from sections of the skin. Negative control slides were done by omitting the primary antibody. A distinct brown nuclear staining was scored positive.

Interpretation of the immunohistochemical expression of c-fos:

A brown nucleus indicated positive expression in the cardiomyocyte of the c-fos oncogene protein. Brown-yellow particles in the cytoplasm indicated positive expression of c-fos oncogene mRNA. The number of positive nuclei of five high-power fields was calculated under light microscope (Zhang et al., 2010). Counting was done in 50 fields and the average was calculated.

STATISTICAL ANALYSIS

All data were expressed as mean value ± standard deviation (SD). To assess statistical significance, Student's t-test was used to compare data between groups. A measured level of $p < 0.05$ was considered significant.

RESULTS

Histopathology :

Figure (1) illustrates few foci of intramyocardial hemorrhage in rats of Group (A1). Figure (2) shows few square nuclei and thrombi in the intramyocardial vessels in rats of Group (A2).

Figures (1, 3, 4) represent histopathological changes detected in group C: in the

form of heamorrhagic areas in the myocardium, many square nuclei and bands of distended myocardial cells alternating with hypercontracted ones (myofibers break-up). Figure (5) shows oval nuclei in control group (D). No histopathological abnormalities could be seen in myocardium of rats of group (B).

Immunohistochemistry:

Table (1) shows immunohistochemical results of c-fos expression in the studied groups presented by mean ±SD. Few c-fos oncogene protein positive cardiomyocyte nuclei are seen in rats of groups (A1) and (B1). Positive expression of c-fos protein increases in rats of groups C1, C2 and A2 (4.1±0.88, 2.7±0.48 and 1.6±0.52) respectively. No c-fos oncogene protein expression is seen neither in control group (D) nor in group (B2). This coincided with the histopathological changes observed, as rats of group (C) are the most affected followed by rats of group (A).

Significant differences ($p<0.001$) in c-fos oncogene protein expression are observed between rats of A1, A2, C1 and C2. Significant differences ($p<0.001$) are also seen between rats of A2, B1 and B2. While less significant differences ($p<0.02$) in c-fos oncogene protein expression are detected between groups B1 and B2.

Figures (6,7,8) display few c-fos expression in group (B1), marked brown nuclei c-fos expression in cardiomyocytes in group (C) and negative c-fos expression in group (D) respectively.

DISCUSSION

Death from electricity is a predominantly physiological process, thus, the post-mortem morphological findings are usually not evident and generally non-specific. The flow of electric current has specific effects on excitable tissues but typical morphological signs may be sparse or even absent (Wang et al., 2008 a). Electric marks are found more frequently with high than low voltage current, and the circumstances may not indicate that electric current has passed through the body. This possible paucity of findings can cause considerable problems in the diagnosis of electrocution (Karger et al., 2002).

Regarding the pathological changes in the cardiac muscle in this study, few square nuclei and thrombi in the intramyocardial vessels were seen in rats of group (A2). Heamorrhagic areas in the myocardium, many obvious square nuclei and bands of distended myocardial cells alternating with hypercontracted ones (myofiber break-up) were displayed in rats of group (C). While group (A1) showed minimal changes; in the form of few foci of intramyocardial hemorrhage when compared to control (group D).

Jisheng (1997) described formation of hypercontraction bands, rupture of intercalated disc, and shortening of myocomere, under electron microscope, in an animal model of cardiac damage after non-fatal electric injury and electrocution. Similarly, in an experimental model designed by Qin et al. (2001), rats were subjected to low voltage current. They observed ultrastructure changes of electrically injured tissues in the form of hypercontraction bands in the myocardium.

Break-up of myocardial fibers was also noticed in the myocardium of 90% of electrocution cases examined by Fineschi et al. (2006 b). The myofiber break-up described could be interpreted as a morphologic counterpart of a terminal dysfunction ending in ventricular fibrillation (VF), giving a structural background to the electrical asynchronous activity and could be induced by the passage of abnormal electrical currents (Baroldi et al., 2005).

In this study, break-up of myocardial fibers was not found in any case electrified after death (B1, B2). This agreed with Baroldi et al. (2005), when they described that it appeared to be vital and was an antemortem change. While, Aggrawal (2002) thought that the myofiber break-up may be perhaps a postmortem change.

Vanderwee et al. (1981) distinguished myofiber fragmentation due to knife motion (sometimes referred to as "chatter") in cutting histological sections from myofiber break-up. They also confirmed that similar changes were never described as part of rigor mortis of the myocardium. While Tomita et al. (2004) described that only slight clumping of nuclear chromatin was observed in the myocardium 1 hr after death and dilation of the sarcoplasmic reticulum and contraction bands were seen ten hours later.

Regarding the immunohistochemical (IHC) results in this study. Few c-fos oncogene protein positive cardiomyocyte nuclei were seen in rats of groups (A1) and (B1), this could be explained as in some cases of cervical dislocation the heart continued to beat sometimes for up to 20 minutes until hypoxia caused arrest (Saukko and Knight, 2004). Positive expression of c-fos protein increased in rats of groups C1, C2 and A2 as c-fos plays an important role in cell response to stimulation and has close relation to injury repair of the molecule (Krukoff et al., 1992 and Zhang et al., 2010).

No c-fos oncogene protein expression was detected in the group (B2), while in (B1) it was few. Wang et al. (2005) observed that the expression of c-fos showed faintness in group of rats electrically injured immediately after death, and was

negative in other rats that were electrified later after death.

Significant differences ($p<0.001$) in c-fos oncogene protein expression were observed between rats of A1,A2,C1 and C2. Also significant differences ($p<0.001$) were seen between rats of A2,B1 and B2. This is in agreement with Wang et al. (2008 b), who found that the levels of c-fos mRNA in the antemortem electrocution group increased significantly compared with that of the postmortem electrocution group.

This study concluded that the classical morphology of the heart remains a gold standard to investigate death due to electrical injury in forensic cases. The immunohistochemical changes can provide additional clue for the diagnosis. This study highlights that c-fos expression can clearly discriminate between antemortem and postmortem electrical injuries. More studies should be carried out for measurement of c-fos in different pathological conditions and different organs, and could be correlated with terminal electrocardiographic recordings.

Table 1 : c-fos expression in different groups

Group	Mean ± SD
A1	0.5±0.48
A2	1.6±0.52
B1	0.3±0.53
B2	0
C1	4.1±0.88
C2	2.7±0.48
D1	0
D2	0

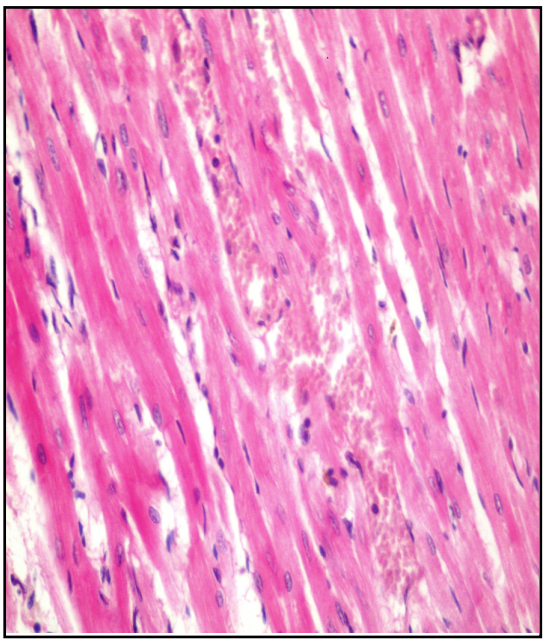

Figure (1) : A photo micrograph of a section from cardiac muscles of groups (A1,C1,C2) showing intramyocardial heamorrhage (H&E X400).

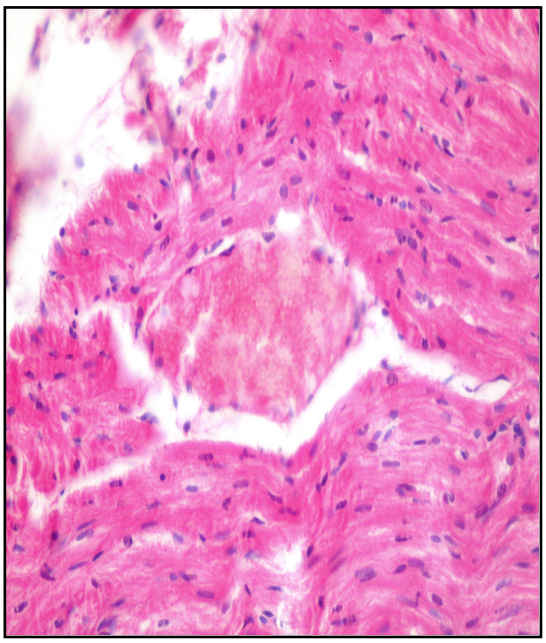

Figure (2) : A photo micrograph of a section from cardiac muscles of group (A2) showing thrombus in intramyocardial vessels (H&E X400).

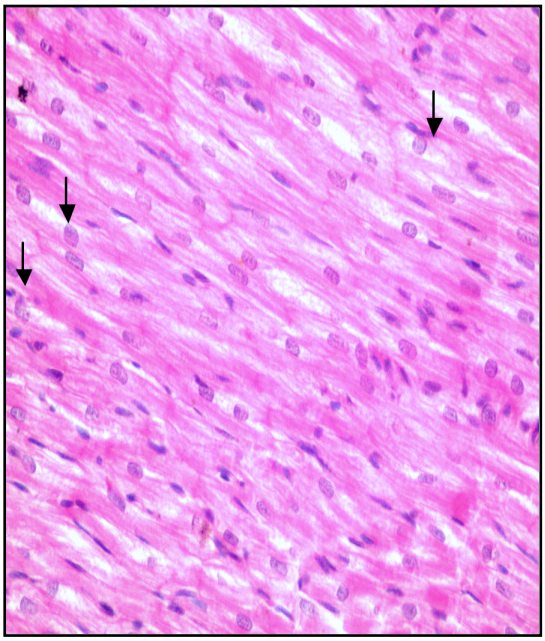

Figure (3) : A photo micrograph of a section from cardiac muscles of groups (C1,C2) showing hypercontracted myocells with many square nuclei (arrows) (H&E X400).

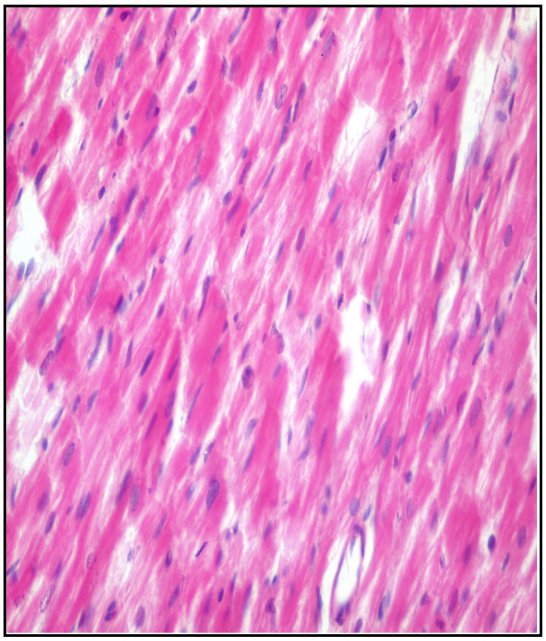

Figure (4) : A photo micrograph of a section from cardiac muscles of groups (C1,C2) showing hypercontracted myocytes alternating with hyper distended cells divided by widened disc (H&E X400).

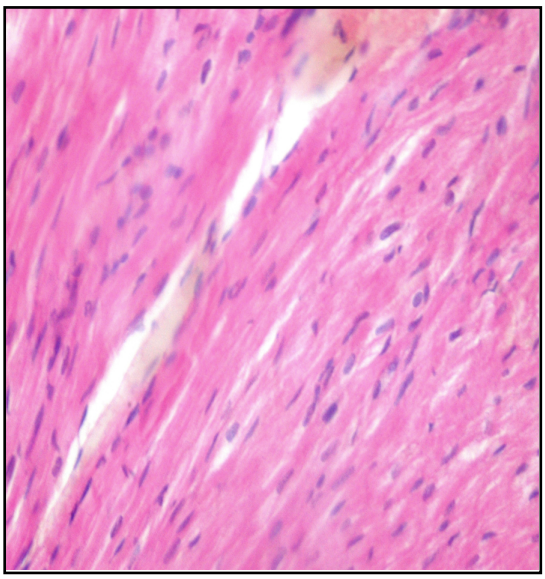

Figure (5) : A photo micrograph of a section from cardiac muscles of control group (D1, D2) showing oval nuclei (H & E X400).

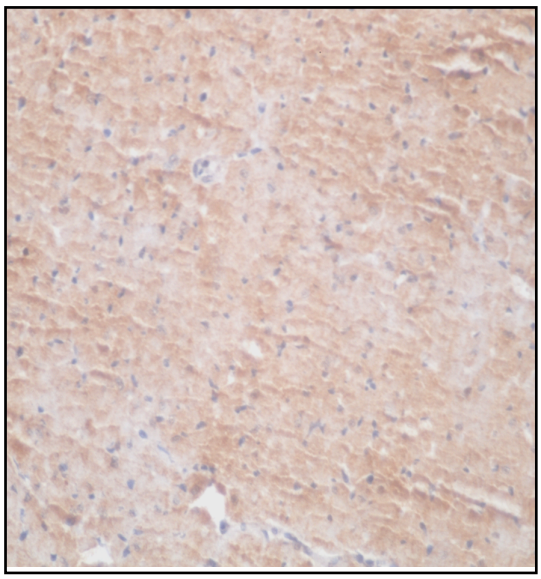

Figure (6) : A photo micrograph of a section from cardiac muscles of group (B1) showing c-fos expression) (IHC X 200).

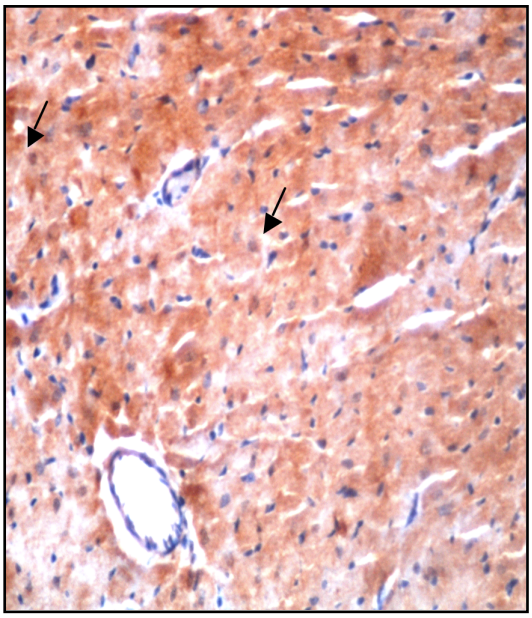

Figure (7) : A photo micrograph of a section from cardiac muscles of groups (C1, C2) showing brown nuclei (arrows) of c-fos expression in cardiomyocytes (IHC x200).

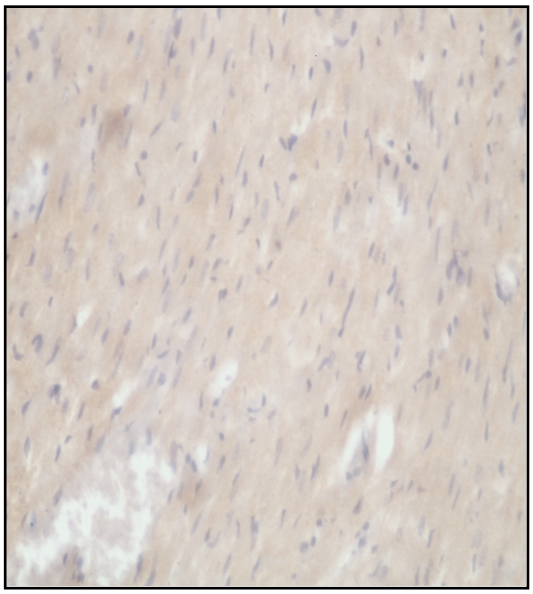

Figure (8) : A photo micrograph of a section from cardiac muscles of control group (D1, D2) showing negative expression of c-fos (IHC X200).

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التغيرات القلبية الهيستوباثولوچية والنسيجية الكيميائية المناعية نتيجة الإصابة بالكهرباء فى الجرذان

المشتركون فى البحث

د. نجوى محمود غندور د. عبير الرفاعى محمد رفاعى* د. غادة على عمران

أقسام الطب الشرعى والسموم الإكلينيكية، والباثولوچيا* - كلية الطب - جامعة أسيوط

تهدف هذه الدراسة إلى الكشف عن التغيرات الهيستوباثولوجية في القلب بعد الإصابة بالكهرباء فى الجرذان والدور المحتمل للچين الورمى c-fos كتعبير مناعي و كمؤشر للتمييز بين الإصابة بالكهرباء قبل و بعد الوفاة.

وقد شملت هذه الدراسة سبعون جزذاً من الإناث تم تقسيمها عشوائياً إلى أربع مجموعات (أ، ب، جـ، د) المجموعة (أ) (٢٠ جزذاً) تعرضت للإصابة بالكهرباء لحظيا قبل الوفاة، ثم قسمت عشوائيا إلى مجموعتين . مجموعة (أ١) حيث تعرضت ١٠ جرذان لخلع العنق على الفور وتم تجميع عينات القلب و مجموعة (أ٢) تعرضت لخلع العنق بعد الإصابة بالكهرباء بساعة واحدة حيث تم تجميع عينات القلب. مجموعة ب (٢٠ جزذاً) تعرضت للإصابة بالكهرباء لحظيا بعد الوفاة عن طريق خلع العنق، وقسمت عشوائيا إلى مجموعتين وجمعت على الفور عينات القلب من ١٠ جرذان مجموعة (ب١) بينما مجموعة (ب٢) أخذت عينات القلب من العشر جرذان الأخرى بعد ساعة واحدة من الإصابة الكهربائية. مجموعة ج (٢٠ جزذاً) صعقت كهربياً حتى الموت، وتنقسم أيضا إلى مجموعتين حيث تم على الفور جمع عينات القلب من مجموعة (ج١) بينما مجموعة (ج٢) أخذت منها عينات القلب بعد مرور ساعة واحدة من الوفاة نتيجة الكهرباء، و أخيراً، ١٠ جرذان تم إستعمالهم كمجموعة ضابطة المجموعة (د) وقسمت عشوائيا إلى مجموعتين كل منها يحتوى على ٥ جرذان مجموعة (د١) وضع لها جهاز الكهرباء ولكن دون تعرضهم لها وتم قتلهم بخلع العنق وجمعت على الفور عينات القلب بينما مجموعة (د٢) جمعت عينات القلب منها بعد ساعة واحدة من خلع العنق ووضع لها أيضاً جهاز الكهرباء بعد الوفاة ولكن دون تعرضهم للصعق، وتم وضع أجزاء من القلوب من كل المجموعات فى الفورمالين ومعالجتها بشكل روتينى وتم الكشف عن الچين الورمى c-fos فى جميع الفئات باستخدام الطريقة الهستوكيميائية المناعية.

أظهرت النتائج أن التغييرات الهستوباثولوجية موجودة بوضوح في مجموعات الجرذان أ، ج وشوهد عدد قليل من الأنوية الايجابية للچين الورمى c-fos في خلايا القلب فى جرذان مجموعة (أ١) وكذلك مجموعة الجرذان (ب١). كما زاد التعبير الإيجابى للبروتين c-fos فى مجموعات الجرذان (أ٢و ج١،٢)، كما كان الكشف عن الچين الورمى للبروتين c-fos سلبياً فى مجموعات الجرذان ب٢، د١،٢ الچين الورمى للبروتين c-fos فى مجموعات الجرذان (ب١، د١، د٢) وكان الكشف الإيجابى عن c-fos ذو دلالة إحصائية بين المجموعات (أ١،٢، ج١،٢).

ولذلك فإن الچين الورمى c-fos يمكن أن يكون مؤشراً لتحديد الإصابة الكهربائية بالإضافة إلى التغييرات الهيستوباثولوجية، كما يمكن استخدامه كدلالة للتمييز بين حالات الإصابة الكهربائية ما إذا كانت قبل أو بعد الوفاة.