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
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...
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 myocose, 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>A1</td>
<td>0.5±0.48</td>
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<tr>
<td>A2</td>
<td>1.6±0.52</td>
</tr>
<tr>
<td>B1</td>
<td>0.3±0.53</td>
</tr>
<tr>
<td>B2</td>
<td>0</td>
</tr>
<tr>
<td>C1</td>
<td>4.1±0.88</td>
</tr>
<tr>
<td>C2</td>
<td>2.7±0.48</td>
</tr>
<tr>
<td>D1</td>
<td>0</td>
</tr>
<tr>
<td>D2</td>
<td>0</td>
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</tbody>
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**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 myocytes 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).
REFERENCES


التغيرات القلبية الهيستوپاثولوجية والنسجية الكيميائية المناعية
نتيجة الإصابة بالكهرباء في الجرذان

المشتركون في البحث

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تهدف هذه الدراسة إلى الكشف عن التغيرات الهيستوپاثولوجية في القلب بعد الإصابة بالكهرباء في الجرذان والدور المحتمل للجين c-fos كتعبير مصاحب ومميز للتصحر بين الإصابة بالكهرباء، قبل وبعد الوفاة.

وقد شملت هذه الدراسة سبعون جزءًا من الإناث تم تقسيمها عشوائيًا إلى أربع مجموعات (أ، ب، ج، د) المجموعة (أ) (20 جزءًا) تعرضت للإصابة بالكهرباء، لاحظياً قبل الوفاة، ثم قسمت عشوائياً إلى مجموعتين، مجموعة (أ1) حيث تعرضت 10 جزءًا لقطع العنق على الفور ثم تجمع عينات القلب و مجموعة (أ2) تعرضت لقطع العنق بعد الإصابة بالكهرباء، بساعة واحدة حيث تم تجميع عينات القلب. مجموعة ب (20 جزءًا) تعرضت للإصابة بالكهرباء، لاحظياً بعد الوفاة عن طريق قطع العنق، وقسمت عشوائياً إلى مجموعتين وجمعوا على الفجر عينات القلب من 10 جزءًا مجموعة (ب1) بينما مجموعة (ب2) أخذت عينات القلب من العشر جزءات الأخرى بعد ساعة واحدة من الإصابة الكهرباء. مجموعة ج (20 جزءًا) صعقت في آلام حتى الموت، وتنقسم أيضًا إلى مجموعتين حيث تم على الفجر جمع عينات القلب من مجموعة (ج1) بينما مجموعة (ج2) أخذت منها عينات القلب بعد مرور ساعة واحدة من الوفاة نتيجة الكهرباء، و أخيرًا، 10 جزءًا تم إستعمالهم كمجموعة ضابطة المجموعة (د) وقسمت عشوائياً إلى مجموعتين كل منها تحتوي على 5 جزءًا مجموعة (د1) ووضع لها جهاز الكهرباء، ولكن دون تعرضهم لها وتكونت بقطع العنق وجمع عينات القلب والغدد عينات القلب و جمعت عينات القلب منها بعد ساعة واحدة من حركة العنق ووضع لها أيضًا جهاز الكهرباء، بعد الوفاة ولكن دون تعرضهم لتصحر، ووضع أخرى من كل المجموعات في الفئران ومعالجتها بشكل روتيني وتم الكشف عن الجين c-fos في جميع الفئات باستخدام الطرق الهيستوپاثولوجية المضافة.

أظهرت النتائج أن التغيرات الهيستوپاثولوجية موجودة ووضوح في المجموعات جدًا، وروتين عدد قليل من الأنواع الإجابة للجين c-fos في خلايا القلب في جزء مجموعة (أ1) وكذلك مجموعة الجرذان (ب1)، كما زاد التعبير الإجابة للبروتين c-fos في مجموعات الجرذان (أ1) عند جزء مجموعة (أ2). كما كان الكشف عن الجين c-fos للبروتين c-fos في مجموعات الجرذان (ب1) و (ب2) وكان الكشف الإجابة عن c-fos في مجموعات (أ1) و (أ2).

وذلك فإن الجين c-fos يمكن أن يكون مؤشراً لتحديد الإصابة الكهرباء بالإضافة إلى التغيرات الهيستوپاثولوجية، كما يمكن استخدامه كدليل للتصحر بين حالات الإصابة الكهرباء ما إذا كانت قبل أو بعد الوفاة.