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Original communication

Heart-type fatty acid binding protein and cardiac troponin I may have a diagnostic value in electrocution: A rat model

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ABSTRACT

Although cardiac injury is known to be the leading cause of death in electrocution, the differential diagnosis can be challenging in forensic practice since the exact mechanism is poorly understood and there is lack of reliable markers. Thus, death due to electrocution may be classified as a negative autopsy. The serum levels of and myocardial immunostaining loss for cardiac troponins and heart-type fatty acid binding protein (H-FABP) are highly sensitive and specific biomarkers of ischemic myocardial damage and may have a diagnostic value in determining the myocardial injury or the cause of death due to electrocution. Due to this reason, a rat model is prepared to investigate these issues. Thirty-two Wistar albino female rats were included and randomly divided into four groups of eight subjects. Group A was the control group, and Group B, C, and D were exposed to electrical current of 110 volt (V), 220 V, and 600 V, respectively. Blood samples and the hearts were collected from the rats for biochemical and immunostaining analyses.

It is found that increased serum H-FABP levels were significantly associated with the higher voltage immediately after electrocution. However, serum cardiac troponin I (cTnI) levels did not show significant changes associated with the higher voltage in the early period of electrocution. As for histopathological examinations, the only significant difference in myocardial immunostaining loss was for H-FABP in Group B.

Serum H-FABP levels may have a diagnostic value in the early postmortem period immediately after electrocution. Besides, it seems that serum H-FABP levels may be a better indicator than those of cTnI to reflect the myocardial damage in the early period of the electrocution.

1. Introduction

Despite performing all the postmortem microscopic and toxicological analyses, the cause of death cannot be determined after some autopsies; this is known as “a negative autopsy”, and it constitutes 1–5% of all of the autopsies.[1–3] Some of the low-voltage electrocution cases may be included in this group because of that macroscopic findings such as the electrical burns occur in only 50% of these cases,[1,2] that it can be difficult to determine the thickness of the burn and the deep tissue damage,[4] and that the majority of injuries and deaths due to electrocution are work-related accidents which additionally create the difficulty to diagnose the cause of death by making the story hidden.

On the other hand, the exact mechanism of the damage during electrocution is poorly understood due to the large number of variables that cannot be measured or controlled. However, cardiac injury is the leading cause of death in electrocution, and research indicate four different mechanisms including the direct effect of current, mechanical trauma, thermal injury, and electro poration.[5–7]

Serum levels of and myocardial immunostaining loss for cardiac troponins (cTns) and the heart-type fatty acid binding protein

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(H-FABP) are highly sensitive and specific biomarkers of ischemic myocardial damage.[8,9] Serum H-FABP levels have been shown to be more sensitive than troponin, myoglobin, or creatine kinase MB (CK–MB) for the diagnosis of acute myocardial infarction (AMI) within 6 h of the onset of chest pain.[10,11] Likewise, autopsy studies of patients who died due to electrocution also showed a significant increase in serum cTn levels.[10,12] However, to the best of our knowledge, serum H-FABP levels have not been investigated to date in electrocution.

Consequently, the levels of cardiac troponins and H-FABP may have a diagnostic value in determining the myocardial injury or the cause of death due to electrocution, may show a different pattern in electrocution than in ischemic myocardial damage, and may contribute to confirm the case history. To investigate these issues, we carried out an animal study. We measured serum levels and immunohistochemical markers of the H-FABP and cardiac troponin I (cTnI) in electrocuted and control rats.

2. Material and methods

This study was approved by the Erciyes University Ethics Committee (Approval Number: 08/33) and carried out in accordance with EU Directive 2010/63/EU for animal experiments at Hakan Çetinsaya Experimental and Clinical Research Center.

2.1. Animals

Thirty-two Wistar albino female rats, each weighing 200–250 g, were included in the study and randomly divided into four groups of eight subjects. Group A was the control group, and Group B, C, and D were exposed to electrical current of 110 volt (V), 220 V, and 600 V, respectively.

2.2. Procedures

Rats were anesthetized intraperitoneally with 60 mg/kg ketamine (Ketalar® Pfizer) and 10 mg/kg of xylazine hydrochloride (Rompun® Bayer). After anesthesia was achieved, two electrodes were placed on the left upper and lower extremities of the rats where the rats in Groups B, C, and D were electrocuted for 5 s later on. Following the electrocution, abdomens were immediately dissected from the midlines, and blood samples were obtained from the abdominal aorta. The chests were dissected to remove the hearts completely, and experimental subjects were then sacrificed. Blood samples were centrifuged to obtain serum for 10 min at 3000 rpm, and the samples were stored at −70 °C until assayed. The heart was washed with saline and fixed in a 10% formaldehyde solution.

Control rats were anesthetized as mentioned above but not electrocuted, although the electrodes were placed on their extremities. The blood samples and the hearts were obtained, and then the rats were sacrificed.

2.3. Biochemical analyses

Serum samples were assayed by ELISA method using cTnI kit (High sensitivity rat cardiac troponin I ELISA kit, Life Diagnostics, Inc., Cat. No. 2010-2-HS) and H-FABP kit (High sensitivity Rat H-FABP ELISA kit, Life Diagnostics, Inc., Cat. No. 2310-2-HSA). Serum H-FABP and cTnI levels were measured as ng/ml and pg/ml, respectively.

2.4. Histopathological analyses

The heart tissues were blocked with paraffin at the Department of Pathology of Medical Faculty, Erciyes University. Snippets that sectioned in thickness of 5 microns were cut out from paraffin blocks by microtome and placed on a slide coated with poly-L-lysine.

Immunohistochemical staining was performed according to manufacturer's protocol and Meng X.[13] Shortly 5 micron-thick sections were deparaffinized, rehydrated in graded alcohols, antigene retrieval was made in citrat buffer in microwave for 20 min at a power of 50%, washed with distillated water after cooling in room temperature, endogenous peroxidase activity was inhibited by incubating in 3% hydrogen peroxidase, washed with distillated water and phosphate buffered saline (PBS), incubated with primary antibody (FABP: Abcam/cardiac FABP antibody/ab16916 or troponin I: Abcam/cardiac troponin I antibody/ab19615) at a dilution of 1/200 for 60 min, washed with PBS, incubated with biotinylated secondary antibody, washed with PBS, incubated with streptavidine peroxidase, washed with PBS, incubated in 3,3′- diaminobenzidine solution, rinsed in distillated water, counterstained with Mayer's hematoxylin, dehydrated with distillated water and graded alcohol, cleared in xylene and coverslipped.

Each immunostained slide was reviewed by two pathologists according to the immunohistochemical evaluation criteria based on previous studies (Table 1).[8,13]

2.5. Statistical analyses

Statistical analyses were performed using SPSS 15.0. Data of the control group and the groups of B, C, and D were analyzed. Normality of data was evaluated using the Shapiro–Wilk test. The Kruskal–Wallis test was used to compare groups with posthoc Tukey's and Student–Newman–Keuls tests. Data were presented as "mean ± standard deviation" and "median (25%–75%)", and $p < 0.05$ was considered as statistically significant.

3. Results

Results from the statistical analyses of serum H-FABP and cTnI levels of the groups are presented in Table 2. Statistically significant differences were observed in the serum H-FABP levels with increasing voltage among the groups, and serum cTnI levels also showed significant differences.

Statistical analyses related to the H-FABP and cTnI immunostaining of the heart tissue of the groups are shown in Table 3. H-FABP immunostaining grades were statistically significantly different among the groups, but cTnI immunostaining grades did not show any difference.

4. Discussion

In the study, we found that increased serum H-FABP levels were significantly associated with the higher voltage immediately after electrocution. However, serum cTnI levels did not show any significant changes associated with the higher voltage in the early period of electrocution. As for histopathological examinations, the only significant difference in the immunostaining loss was for H-FABP in Group B.

4.1. cTnI findings

Our findings of serum cTnI levels have shown that serum cTnI levels may not be a reliable indicator of the heart damage in the early stages of electrocution, although increased postmortem levels