Comet assay in evaluating deoxyribonucleic acid damage after out-of-hospital cardiac arrest

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Objective: This study aimed to investigate whether out-of-hospital cardiac arrest (OHCA) may induce severe DNA damage measured using comet assay in successfully resuscitated humans and to evaluate a short-term prognostic role.

Methods: In this prospective, controlled, blinded study (1/2013–1/2014), 41 patients (age, 63±14 years) successfully resuscitated from non-traumatic OHCA and 10 healthy controls (age, 53±17 years) were enrolled. DNA damage [double-strand breaks (DSBs) and single-strand breaks (SSBs)] was measured using comet assay in peripheral lymphocytes sampled at admission. Clinical data were recorded (according to Utstein style). A good short-term prognosis was defined as survival for 30 days.

Results: Among the patients, there were 71% (29/41) short-term survivors. After OHCA, DNA damage (DSBs and SSBs) was higher (11.0±7.6% and 0.79±2.41% in tail) among patients than among controls (1.96±1.63% and 0.02±0.03% in tail), and it was more apparent for DSBs (p<0.001 and p=0.085). There was no difference in the DNA damage between patients with cardiac and non-cardiac etiology, or between survivors and non-survivors. Among Utstein style parameters, ventricular fibrillation, asystole, and early electrical defibrillation influenced DSBs; none of the factors influenced SSBs. Factors influencing survival were SSBs, ventricular fibrillation, length of cardiopulmonary resuscitation by professionals ≤15 min, cardiogenic shock, and postanoxic encephalopathy. In contrast to DSBs [area under the curve (AUC)=0.520], SSBs seem to have a potential in prognostication (AUC=0.639).

Conclusion: This study for the first time demonstrates revelation of DNA damage using comet assay in patients successfully resuscitated from OHCA. Whether DNA damage measured using comet assay may be a prognostic marker remains unknown, although our data may encourage some suggestions. (Anatol J Cardiol 2017; 18: 31-8)

Keywords: cardiac arrest, out-of-hospital, DNA damage, comet assay, cardiopulmonary resuscitation, survivors

Introduction

There is a great emphasis on the need for early prognostication (at emergency room or before) of patients after out-of-hospital cardiac arrest (OHCA). Optimal early prognostic markers should be independent on both sufficient time for neurological recovery and major clinical status confounders (sedation, neuromuscular blockade, and metabolic derangements) (1). Early prognostic markers may be reliable only when a standardized, evidence-based post-cardiopulmonary resuscitation (CPR) treatment care is assumed. Prognostication is now based on a multimodal algorithm applied at ≥48 and ≥120 h after OHCA (1). However, the role of DNA integrity in prognostication of patients after OHCA remains unknown (2).

We aimed to investigate whether OHCA may induce severe DNA damage [single-strand breaks (SSBs) and double-strand breaks (DSBs)] measured using comet assay in successfully resuscitated humans and evaluate an OHCA short-term prognosis (30-day survival) using these DNA damage markers.

Methods

Design

This was a prospective, monocentric, controlled, blinded study (1/2013–1/2014). Inclusion and exclusion criteria have been previously reported (2). The patients enrolled in the study were all consecutive adults (age ≥18 years) who were successfully
resuscitated [return of spontaneous circulation within 30 min, survival for ≥60 min following arrival at the emergency department (ED)] by professionals from non-traumatic OHCA of either cardiac or non-cardiac etiology (Table 1) and who met none of the exclusion criteria [active malignancy, the terminal phase of a chronic illness, toxic or suicidal causes (including drowning cases), chemotherapy or radiotherapy within the last year, and X-ray investigation within the last month or before the blood for DNA analysis was sampled). Healthy controls (all 10 consecutive volunteers) were also enrolled. Data were collected according to the Utstein style (3).

**Ethics**

The study complied with the principles of the 1975 Declaration of Helsinki, and the local Ethics Committee approved the study protocol. Patients’ written informed consents were resolved with the aid of the law courts. Controls gave their written informed consents.

**Patient group**

A total of 41 patients [28 men; aged 63 (34–88), 63±14 years] together with 10 healthy controls [5 men; aged 55 (20–75), 53±17 years] were tested at admission for DNA damage (DSBs and SSBs) in peripheral lymphocytes using comet assay (Table 1). The majority of OHCA (78% [32/41]) was of cardiac etiology (Fig. 1, Table 1).

**DNA analysis–comet assay**

Peripheral blood samples for comet analysis were collected during the first 15 min (3 mL) after patients were transported to ED and always prior to X-ray examination or the commencement of therapeutic hypothermia. Heparinized venous blood was immediately processed for comet assay starting with lymphocyte isolation (using Histopaque 1077 (3 mL; Sigma-Aldrich, St. Luis, USA); centrifugation (at 400 G for 30 min at 20°C)). Lymphocytes (the white ring on the surface of red cells) were washed (using phosphate buffered saline (PBS), 5 mL; three times), counted, and diluted to a concentration of 105 cells/mL.

DNA damage was measured using both alkaline and neutral versions of the comet assay (4). The alkaline version suitable for SSB detection has been described previously (5, 6). The neutral version suitable for DSB detection was a slight modification of that described in the papers of Olive et al. (7).

Briefly, cells embedded in 1% agarose (Sigma-Aldrich, St. Luís, USA) on microscope slides were lysed overnight at 4°C [1% Triton X-100 (Merck, Dermstadt, Germany), 2 500 mmol/L NaCl (Penta, Praha, Czech Republic, pH 10.0), 100 mmol/L EDTA (Penta, Praha, Czech Republic), and 10 mmol/L Tris (Penta, Praha, Czech Republic)]. Electrophoresis in the alkaline buffer (300 mmol/L NaOH; 1 mmol/L EDTA) was performed at 40 V, 300 mA for 30 min at 4°C after a 40-min period of unwinding. Electrophoresis in the neutral borate buffer (90 mmol/L Tris, 90 mmol/L boric acid, and 2 mmol/L EDTA; pH 8) was performed at 29 V, 6 mA for 40 min at 4°C.

| Pre-hospital characteristics | no. (%) |
|-----------------------------|---------|
| **Long-term medication before OHCA** |         |
| Diuretics                    | 12 (29) |
| Beta-blockers                | 12 (29) |
| ACE inhibitors               | 19 (46) |
| Cigarette smoking            | 14 (34) |
| **Initial cardiac rhythm**   |         |
| Ventricular fibrillation     | 24 (59) |
| Asystole                     | 10 (24) |
| Third-degree atrioventricular block | 1 (2)  |
| Pulseless electrical activity | 6 (15)  |
| **Location**                 |         |
| Home                         | 22 (54) |
| Public place (out of home)   | 19 (46) |
| Early electrical defibrillation | 23 (56) |
| Arrest witnessed             | 34 (83) |
| Bystander CPR                | 28 (68) |
| **Arrival time, min, call–ambulance arrival** |         |
| ≤5 min                       | 14 (34) |
| >5 min                       | 27 (66) |
| **Length of CPR by health care professionals** |         |
| 0–15 min                     | 17 (42) |
| 16–30 min                    | 24 (59) |
| **Hospital characteristics** | no. (%) |
| **Cardiac OHCA etiology**    |         |
| IHD without acute myocardial infarction | 12 (29) |
| IHD acute myocardial infarction | 11 (27) |
| Dilated cardiomyopathy       | 3 (7)   |
| Idiopathic arrhythmia        | 3 (7)   |
| Pulmonary embolism           | 2 (5)   |
| Aortic dissection            | 1 (2)   |
| **Non-cardiac OHCA etiology** |         |
| Pneumonia                    | 5 (12)  |
| Stroke                       | 3 (7)   |
| Anaphylactic shock           | 1 (2)   |
| **Glasgow coma scale at admission** |     |
| 3                            | 37 (90) |
| 4–5                          | 2 (5)   |
| ≥6                           | 2 (5)   |
| STEMI                        | 10 (24) |
| Primary PCI                  | 9 (22)  |
| Cardiogenic shock            | 15 (37) |
| Postanoxic encephalopathy    | 25 (61) |
| Left ventricular ejection fraction ≤ 35% (ECHO) | 10 (24) |
| Emergent coronarography      | 20 (49) |

ACE inhibitors - angiotensin-converting enzyme inhibitors; CPR - cardiopulmonary resuscitation; ECHO, echocardiography; IHD, ischemic heart disease; OHCA, out-of-hospital cardiac arrest; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction.
after washing out the borate buffer (three times). After neutralization (three times for 5 min; 400 mmol/L Tris–HCl, pH 7.5; once in distilled water), drying overnight on filter paper, and staining (ethidium bromide, 10 μL; 2.5 mmol/L; Sigma-Aldrich, St. Luis, USA), both comet versions were analyzed using fluorescence microscopy (excitation filter of 450–490 nm; suppression filter LP, 520 nm; 200× magnification). One hundred randomly selected lymphocytes were examined using the comet module of Lucia G image analysis (Laboratory Imaging, Prague, Czech Republic). Fifty cells per slide and two slides per patient were analyzed. The fluorescence intensity of the comet tail (DNA breaks) relative to the head (intact DNA) reflects the number of DNA breaks, expressed as the “percentage of DNA in comet tail” (% in tail). For each individual patient, the median from all values of “% in tail” were used.

Upper levels of normal ranges of DNA breaks (DSBs or SSBs) were calculated from parameters of healthy controls (as more than “mean±2 SD”: for DSBs >5.22% in tail and for SSBs >0.08% in tail). % in tail-percentage of DNA in comet tail

Results

Among 41 patients (Table 1, Fig. 1), there were 71% (29/41) survivors at day 30. The proportion of patients discharged alive was equal (71%). The length of hospitalization was 12 (1; 107); 18±20 days.

Double-strand breaks

The frequency of DSBs at pathological level has been shown in Figure 1. DSBs values and differences in these values between subgroups are presented in Figure 2a. Factors influencing DSBs were ventricular fibrillation and early electrical defibrillation, which lowered the number of DSBs and asystole (which increased the number of DSBs) (Table 2). The optimal cut-off value for prediction of short-term survival based on DSBs was 15.1% in tail (Fig. 3a).

Single-strand breaks

The pathological level for frequency of SSBs has been shown in Figure 1. SSBs values and differences in these values between subgroups are presented in Figure 2b. No factors influencing SSBs were found (Table 2). The optimal cut-off value for prediction of short-term survival based on SSBs was 0.15% in tail (Fig. 3b).
Outcomes

Four factors including SSBs, ventricular fibrillation, length of CPR ≤ 15 min by health care professionals, and cardiogenic shock were found to be predictive of 30-day survival; no predictive value was found for other factors including DSBs (Table 3).

In contrast to DSBs (Fig. 3a; AUC=0.520), SSBs have a prognostic value (Fig. 3b; AUC=0.639).

Discussion

In the present study, the priority was to test the genotoxic effect of OHCA on intact and easily-available cells at admission and to evaluate the short-term prognostic role of induced genotoxicity (DNA damage: SSBs and DSBs) using comet assay. According to the findings of the present study, DNA damage was present in patients successfully resuscitated from OHCA.
Whether DNA damage is a prognostic marker remains unknown. There are huge advances in acute care medicine, nevertheless the prognosis of patients after OHCA remains poor (8–10). The management of resuscitated patients is a very pressing issue, not only medically but also economically and ethically. Hence, it remains a clinical challenge to find an early predictor of prognosis after OHCA to facilitate reliable patient triage.

Despite the existence of several papers on DNA damage in cardiac disease (11–20), currently, very few papers have been published on genomic markers during and after OHCA (2, 21–24). White et al. (21) tested DNA from the cerebral cortex of dogs during their reperfusion following resuscitation for cardiac arrest, but no significant damage was found. However, a crucial limitation is that any study of DNA integrity in the cerebral cortex is inapplicable for clinical practice. In humans, the prognostic value of plasma cell-free DNA has recently been studied in patients after OHCA (22–24). Despite the small cohorts (81, 67, and 42 cases), the plasma cell-free DNA level detected using real-time polymerase chain reaction assay has been presented as a promising independent predictor of 24-h in-hospital mortality after OHCA (22–24). Unfortunately, the recommendation of the abovementioned studies is to collect samples for cell-free DNA level detection at 24 h after admission. We suggest that this is quite late for an early patient triage. Admittedly, it is compensated for by a slightly higher predictive value for 24-h hospital mortality (AUC=0.762) when comparing predictions based on samples provided at admission (AUC=0.636) (23). An additional limitation for clinical practice is that the major source of cell-free DNA circulating in the bloodstream are irreversibly destroyed cells, which are unable to reverse this unfavorable state.

In contrast to these works, our results clearly demonstrated OHCA genotoxicity (DNA damage) in intact cells, which is detectable prior to the destruction of the cells, and thus preserve the prospect for reversibility in case of appropriate therapeutic interventions. In a previous study, we detected DNA damage

| Factors (yes versus no) | DSBs | DSBs | P  | SSBs | SSBs | P  |
|-------------------------|------|------|----|------|------|----|
|                         | Median | 95% CI | Median | 95% CI |     | Median | 95% CI | Median | 95% CI |     |
| Men                     | 10.8 | 5.3–14.2 | 8.0 | 2.0–15.0 | 0.39 | 0.04 | 0–0.15 | 0.05 | 0–0.37 | 0.89 |
| Age ≥70 years           | 10.1 | 7.5–15.1 | 10.2 | 4.1–14.2 | 0.55 | 0.02 | 0–0.37 | 0.045 | 0.02–0.16 | 0.55 |
| Diuretics               | 12.7 | 10.1–20.0 | 9.74 | 3.0–11.4 | 0.09 | 0.085 | 0.01–0.36 | 0.04 | 0–0.16 | 0.80 |
| Beta-blockers           | 13.9 | 4.1–18.4 | 9.8 | 5.0–11.4 | 0.39 | 0.08 | 0–0.19 | 0.04 | 0–0.16 | 0.45 |
| ACE inhibitors          | 11.3 | 4.4–16.3 | 9.8 | 3.0–14.2 | 0.32 | 0.02 | 0–0.15 | 0.045 | 0–0.19 | 0.68 |
| Cigarette smoking       | 10.8 | 3.0–14.2 | 9.74 | 4.4–15.0 | 0.97 | 0.045 | 0–0.53 | 0.04 | 0–0.15 | 0.87 |
| Ventricular fibrillation| 9.7 | 4.1–11.4 | 12.7 | 8.0–22.6 | 0.04 | 0.045 | 0–0.19 | 0.04 | 0.01–0.14 | 0.82 |
| Asystole                | 12.8 | 4.4–24.5 | 9.8 | 5.0–12.7 | 0.05 | 0.05 | 0–0.36 | 0.04 | 0–0.16 | 1.00 |
| Location of arrest, home| 7.8 | 2.2–10.9 | 14.2 | 9.7–16.3 | 0.07 | 0.05 | 0–0.36 | 0.04 | 0–0.11 | 0.52 |
| Early electrical defibrillation | 9.7 | 4.1–11.4 | 12.7 | 8.0–22.6 | 0.04 | 0.04 | 0–0.30 | 0.045 | 0.01–0.15 | 0.88 |
| Arrest witnessed        | 10.4 | 6.7–12.7 | 10.1 | 0.2–24.5 | 0.98 | 0.05 | 0.02–0.16 | 0 | 0–0.36 | 0.42 |
| Bystander CPR           | 10.0 | 4.1–12.7 | 10.7 | 4.4–22.7 | 0.74 | 0.04 | 0–0.15 | 0.06 | 0.01–0.32 | 0.69 |
| Arrival time, ≤5 min    | 12.8 | 2.0–16.3 | 10.0 | 6.7–12.7 | 1.00 | 0.03 | 0–0.19 | 0.05 | 0–0.16 | 0.75 |
| Length of CPR by health care professionals, ≤15 min | 11.4 | 4.1–15.0 | 9.9 | 5.0–15.0 | 0.81 | 0.15 | 0.01–0.53 | 0.04 | 0–0.09 | 0.22 |
| Hypokalemia at admission| 13.1 | 8.0–18.4 | 9.7 | 4.1–12.7 | 0.26 | 0.075 | 0–0.19 | 0.04 | 0–0.30 | 0.75 |
| STEMI                   | 8.4 | 1.8–24.5 | 10.7 | 7.5–15.0 | 0.78 | 0.02 | 0–0.83 | 0.05 | 0.02–0.16 | 0.64 |
| Cardiogenic shock       | 9.7 | 5.0–20.0 | 11.1 | 3.0–14.2 | 0.56 | 0.09 | 0.02–0.37 | 0.04 | 0–0.11 | 0.24 |
| Postanoxic encephalopathy| 10.0 | 5.3–15.0 | 10.8 | 2.2–15.0 | 0.64 | 0.04 | 0–0.14 | 0.085 | 0.01–0.30 | 0.32 |
| Left ventricular EF ≤35%| 10.0 | 5.0–18.4 | 10.9 | 4.4–15.0 | 0.94 | 0.055 | 0–0.19 | 0.04 | 0–0.30 | 0.73 |
| Emergent coronarography | 8.9 | 5.0–11.4 | 12.7 | 4.1–17.4 | 0.28 | 0.125 | 0.02–0.37 | 0.02 | 0–0.09 | 0.21 |
| Cardiac etiology of OHCA| 9.9 | 5.0–12.7 | 12.7 | 2.8–22.7 | 0.31 | 0.05 | 0.02–0.16 | 0.04 | 0–0.32 | 0.69 |
| Survivors               | 10.9 | 5.3–15.0 | 9.9 | 4.4–15.0 | 0.82 | 0.06 | 0.10–0.30 | 0.03 | 0–0.09 | 0.16 |

ACE inhibitors - angiotensin-converting enzyme inhibitors; CI - confidence interval; CPR - cardiopulmonary resuscitation; DSBs - double-strand breaks (% of DNA in comet tail); EF - ejection fraction; OHCA - out-of-hospital cardiac arrest; P-value of significance <0.05; SSBs - single-strand breaks (% of DNA in comet tail); STEMI - ST-segment elevation myocardial infarction; Two-sample t-test or nonparametric Mann–Whitney U test.
When comparing cell-free DNA, the authors assumed based of pathophysiology that the optimal time at which analysis of blood samples should be performed for DNA damage should be shortly (minutes or hours) after OHCA. Unfortunately, serial measurements of DNA damage after OHCA have not been provided in our work. It was shown by others (25, 26) that maximum DNA changes occur 60–180 min after exposure to some factors (namely isoflurane anesthesia); therefore, it might be beneficial to sample the blood in OHCA patients later than at admission (our study) to get better predictive results. Also, Corbucci et al. (27) detected still-rising DNA damage during 100 min of controlled ischemia-reperfusion exposure. Unfortunately, the DNA damage was measured in human cardiac myocytes, which are unavailable material, similarly to a study by Bartunek et al. (27, 28). On the other hand, using this protocol with samples collected 60–180 minutes after OHCA may be rendered invalid in practice because of exposure to other diagnostic and therapeutic factors, which may induce DNA damage (e.g., X-ray; the routine practice usually includes emergent angiography or computer tomography).

However, at admission, both DNA breaks and cell-free DNA give comparable predictive values of hospital mortality (AUC for SSBs with comet assay: 0.639; DSBs with comet assay: 0.520; γH2AX: 0.602; cell-free DNA: 0.636) (2, 23). For appropriate interpretation, one should be aware that Gornik et al. (23) calculated the predictive power from 24-h mortality, not from in-hospital mortality, despite there being an apparent difference between the two (24-h mortality, 37% vs. in-hospital mortality, 72%; the length of hospitalization in Gornik’s study was not specified) (23). In our study, the predictive value was calculated from 30-day mortality (32%). Among the Utstein style parameters (3), the differences in DSB results (comet assay) were applied to three factors (ventricular fibrillation as the initial rhythm, early electrical defibrillation resulting in lower DSBs, and asystole as the initial rhythm increasing the number of DSBs), while in the γH2AX method, the difference was applied to the initial rhythm (2). In contrast to DSBs, no differences were found at this point in SSBs (comet assay).

Analysis of the association between survival at day 30 association and the Utstein style factors was in our small study the only marginal matter. Nevertheless, our results concur with the literature: the direct association between 30-day survival and ventricular fibrillation or length of CPR by health care professionals ≤ 15 min and the indirect association between 30-day survival and cardiogenic shock. However, this study is the first to describe the direct association between survival at day 30 and SSBs.

The prognostic role of DNA damage in patients successfully resuscitated from OHCA remains unknown. Despite this, our study suggests a useful prognostic potential for DNA damage analyzed using either comet assay or the γH2AX method (AUC, 0.520–0.639) (2). These results should be verified in a future study including serial measurements and comparison with cell-free DNA results (22–24).

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**Table 3. Analysis of risk factors for survival (univariate logistic regression)**

| Factors (yes versus no) | OR   | R²   | 95% CI    | P    | %    |
|------------------------|------|------|-----------|------|------|
| DSBs>optimal cut-off   | 1.90 | 0.075| 0.34;10.7 | 0.447| 44   |
| SSBs>optimal cut-off   | 7.76 | 0.440| 0.88;68.4 | 0.025| 56   |
| Men                    | 3.14 | 0.273| 0.76;13.0 | 0.112| 68   |
| Age ≥70 years          | 0.32 | 0.273| 0.08;13.1 | 0.112| 68   |
| Diuretics              | 0.76 | 0.018| 0.18;3.25 | 0.715| 61   |
| Beta-blockers          | 1.35 | 0.021| 0.29;6.20 | 0.697| 44   |
| ACE inhibitors         | 1.31 | 0.020| 0.34;5.09 | 0.699| 51   |
| Cigarette smoking      | 1.05 | 0.001| 0.25;4.37 | 0.944| 44   |
| Ventricular fibrillation| 4.44 | 0.411| 1.06;18.7 | 0.035| 68   |
| Asystole               | 0.52 | 0.091| 0.12;2.34 | 0.400| 66   |
| Location of arrest, home | 1.23 | 0.012| 0.32;4.74 | 0.763| 54   |
| Early electrical defibrillation | 3.8  | 0.354| 0.92;15.8 | 0.058| 66   |
| Arrest witnessed       | 4.33 | 0.303| 0.80;23.6 | 0.088| 73   |
| Bystander CPR          | 3.14 | 0.273| 0.76;13.0 | 0.112| 68   |
| Arrival time, ≤5 min   | 0.63 | 0.055| 0.16;2.53 | 0.517| 61   |
| Length of CPR by health care professionals, ≤15 min | 13.54 | 0.614| 1.54;119  | 0.003| 66   |
| Hypokalemia at admission | 4.95 | 0.290| 0.55;44.4 | 0.098| 49   |
| X-ray                  | 1.23 | 0.012| 0.32;4.74 | 0.763| 54   |
| STEMI                  | 0.52 | 0.091| 0.12;2.34 | 0.400| 66   |
| Cardiogenic shock      | 0.042| 0.798| 0.007;0.25 | 0.001| 83   |
| Postanoxic encephalopathy | Calculation has failed | | | | |
| Left ventricular EF ≤35% | 0.52 | 0.091| 0.12;2.34 | 0.400| 66   |
| Emergent coronarography | 0.93  | 0.001| 0.24;3.58 | 0.920| 51   |

ACE inhibitors - angiotensin-converting enzyme inhibitors; CI - confidence interval; CPR - cardiopulmonary resuscitation; DSBs - double-strand breaks (comet assay); DSBs>optimal cut-off, value of DSBs higher than receiver operating curve optimal cut-off value, which is 15.1% of deoxyribonucleic acid in tail; EF - ejection fraction; OR - odds ratio; %, percent of the true classification; R² - the proportion of variation in the dependent variable accounted for by the independent variables; SSBs - single-strand breaks (comet assay); SSBs>optimal cut-off, value of SSBs higher than receiver operating curve optimal cut-off value, which is 0.15% of deoxyribonucleic acid in comet tail; STEMI - ST-segment elevation myocardial infarction; Results are employed for survival (thus OR<0 indicates a lower chance for survival).
Based on our results, we hypothesize that DNA damage (assessed using comet assay or the γH2AX method) is a more sensitive marker for post-resuscitation outcomes when compared with the cell-free DNA level.

**Study limitations**

The present study has several methodological limitations. The primary limitation is that it was a single-center study with a small number of participants, which is a consequence of the prospective and pilot design of the study. Another limitation is that the control group was not matched to the cohort. Despite that, the authors judge the control group to be adequate because numerous literature data confirmed that under normal conditions, lymphocytes show a low background level of SSBs or DSBs (29). The main question, which our results have opened up, is regarding the best timing for collection of DNA damage samples.

**Conclusion**

In conclusion, our study demonstrated significant DNA damage, especially for DSBs, measured using comet assay in patients successfully resuscitated from OHCA compared with that in controls with no dependency on the cardiac arrest etiology.

The prognostic value of DNA damage remains unknown, although our results suggest a potential usefulness. Future research should include serial measurements of DNA damage at admission and later to test the influence of DNA damage dynamics on post-arrest patient outcomes.

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**References**

1. Nolan JP, Soar J, Cariou A, Cronberg T, Moulael V, Deakin CD, et al. European Resuscitation Council and European Society of Intensive Care Medicine Guidelines for Post-resuscitation Care 2015: Section 5 of the European Resuscitation Council Guidelines for Resuscitation 2015. Resuscitation 2015; 95: 202-22.

2. Hazuková R, Režáčová M, Kočí J, Čermáková E, Pleskot M. Severe deoxyribonucleic acid damage after out-of-hospital cardiac arrest in successfully resuscitated humans. Int J Cardiol 2016; 207: 33-5.

3. Jacobs I, Nadkarni V, Bah J, Berg RA, Billi JE, Bossaert L, et al; International Liaison Committee on Resuscitation. Cardiac arrest and cardiopulmonary resuscitation outcome reports: update and simplification of the Utstein templates for resuscitation registries. A statement for healthcare professionals from a task force of the international liaison committee on resuscitation (American Heart Association, European Resuscitation Council, Australian Resuscitation Council, New Zealand Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Southern Africa). Resuscitation 2004; 63: 233-49.

4. Cmielova J, Havelek R, Kohlerova R, Soukup T, Bruckova L, Suchanek J, et al. The effect of ATM kinase inhibition on the initial response of human dental pulp and periodontal ligament mesenchymal stem cells to ionizing radiation. Int J Radiat Biol 2013; 89: 501-11.

5. Collins AR, Dušinská M, Gedik CM, Štětina R. Oxidative damage to DNA: do we have a reliable biomarker? Environ Health Perspect 1996; 104 (Suppl. 3): 465-9.

6. Collins AR, Dobson VL, Dušinská M, Kennedy G, Štětina R. The comet assay: what can it really tell us? Mutat Res 1997; 375: 183-93.

7. Olive PL, Wlodek D, Banáth JP. DNA double-strand breaks measured in individual cells subjected to gel electrophoresis. Cancer Res 1991; 51: 4671-6.

8. Strömsöe A, Svensson L, Axelsson ÅB, Claesson A, Grönnös K, Nordberg P et al. Improved outcome in Sweden after out-of-hospital cardiac arrest and possible association with improvements in every link in the chain of survival. Eur Heart J 2015; 36: 863-71.

9. Sasson C, Rogers MA, Dahl J, Kellerman AL. Predictors of survival from out-of-hospital cardiac arrest: a systematic review and meta-analysis. Circ Cardiovasc Qual Outcomes 2010; 3: 63-81.

10. Daya MR, Schmicker RH, Zive DM, Rea TD, Nichol G, Buick JE, et al; Resuscitation Outcomes Consortium Investigators. Out-of-hospital cardiac arrest survival improving over time: Results from the Resuscitation Outcomes Consortium. Resuscitation 2015; 91: 108-15.

11. Bhat MA, Mahajan N, Gandhi G. DNA and chromosomal damage in coronary artery disease patients. EXCLI J 2013; 12: 872-84.

12. Yıldız A, Gür M, Yılmaz R, Demirbağ R, Çelik H, Aslan M, et al. Lymphocyte DNA damage and total antioxidant status in patients with white-coat hypertension and sustained hypertension. Turk Kardiyol Dern Ars 2008; 36: 231-8.

13. Demirbağ R, Yılmaz R, Koçyiğit A, Güzel S. Effect of coronary angiography on oxidative DNA damage observed in circulating lymphocytes. Angiology 2007; 58: 141-7.

14. Gür M, Yılmaz R, Demirbağ R, Yıldız A, Koçyiğit A, Çelik H, et al. Lymphocyte DNA damage is associated with increased aortic intima-media thickness. Mutat Res 2007; 617: 111-8.

15. Demirbağ R, Yılmaz R, Gür M, Çelik H, Güzel S, Selek S, et al. DNA damage in metabolic syndrome and its associations with antioxidative and oxidative measurements. Int J Clin Pract 2006; 60: 1187-93.

16. Demirbağ R, Yılmaz R, Güzel S, Çelik H, Koçyiğit A, Özcan E. Effects of treadmill exercise test on oxidative/antioxidative parameters and DNA damage. Anadolu Kardiyol Derg 2006; 6: 135-40.

17. Demirbağ R, Yılmaz R, Gür M, Koçyiğit A, Çelik H, Güzel S, et al. Lymphocyte DNA damage in patients with acute coronary syndrome and its relationship with severity of acute coronary syndrome. Mutat Res 2005; 578: 298-307.

18. Karahalil B, Polat S, Senköy A, Bölükbaşi S. Evaluation of DNA damage after tourniquet-induced ischaemia/reperfusion injury during lower extremity surgery. Injury 2010; 41: 758-62.
19. Karahalil B, Gümüş T, Emerce E, İzdeş S, Kanbak O, Kesimci E. Comet assay in evaluating DNA damage associated with ischaemia-reperfusion injury in patients undergoing coronary surgery. Arh Hig Rada Toksikol 2009; 60: 307-15.
20. G V, HYS, Bhat BV, Chand P, Rao KR. Hypoxia induced DNA damage in children with isolated septal defect and septal defect with great vessel anomaly of heart. J Clin Diagn Res 2014; 8: SC01-3.
21. White BC, DeGracia DJ, Krause GS, Skjaerlund JM, O’Neil BJ, Grossman LI. Brain nuclear DNA survives cardiac arrest and reperfusion. Free Radic Biol Med 1991; 10: 125-35.
22. Arnalich F, Menéndez M, Lagos V, Ciria E, Quesada A, Codoceo R, et al. Prognostic value of cell-free plasma DNA in patients with cardiac arrest outside the hospital: an observational cohort study. Crit Care 2010; 14: R47.
23. Gornik I, Wagner J, Gašparović V, Miličić D, Degoricija V, Skorić B, et al. Prognostic value of cell-free DNA in plasma of out-of-hospital cardiac arrest survivors at ICU admission and 24h post-admission. Resuscitation 2014; 85: 233-7.
24. Huang CH, Tsai MS, Hsu CY, Chen HW, Wang TD, Chang WT, et al. Circulating cell-free DNA levels correlate with postresuscitation survival rates in out-of-hospital cardiac arrest patients. Resuscitation 2012; 83: 213-8.
25. Reitz M, Antonini-Rumpf E, Lanz E. DNA single strand breaks in peripheral human lymphocytes after anesthesia with isoflurane-nitrous oxide-oxygen. Arzneimittelforschung 1993; 43: 1258-61.
26. Şardaş S, Karabiyik L, Aygün N, Karakaya AE. DNA damage evaluated by the alkaline comet assay in lymphocytes of humans anaesthetized with isoflurane. Mutat Res 1998; 418: 1-6.
27. Corbucci GG, Perrino C, Donato G, Ricchi A, Lettieri B, Troncone G, et al. Transient and reversible deoxyribonucleic acid damage in human left ventricle under controlled ischemia and reperfusion. J Am Coll Cardiol 2004; 43: 1992-9.
28. Bartunek J, Vanderheyden M, Knaapen MW, Tack W, Kockx MM, Goethals M. Deoxyribonucleic acid damage/repair proteins are elevated in the failing human myocardium due to idiopathic dilated cardiomyopathy. J Am Coll Cardiol 2002; 40: 1097-103.
29. Andreassi MG, Botto N, Rizza A, Colombo MG, Palmieri C, Berti S, et al. Deoxyribonucleic acid damage in human lymphocytes after percutaneous transluminal coronary angioplasty. J Am Coll Cardiol 2002; 40: 862-8.