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Research Article

# Plasma mitochondrial DNA at admission can predict the outcome of acute trauma patients admitted to ICU

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**KEYWORDS**

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**Abstract** *Objectives:* We assessed whether at admission plasma circulating cell free mitochondrial DNA (ccf-mtDNA) is related to injury severity and can predict morbidity and mortality in acute trauma patients.

*Patients and methods:* Patients were evaluated at Emergency Department (ED) using Injury Severity Scale (ISS), but only patients required ICU admission were studied (Group B). At ED arrival, blood samples were obtained for quantitative real-time PCR estimation of plasma level of ccf-mtDNA. Study outcome was the correlation between morbidity and mortality and at admission plasma ccf-mtDNA level and its predictability for morbidity and mortality. Ten healthy volunteers gave blood samples as control group (Group B).

*Results:* Twenty-seven patients passed smooth ICU stay and were discharged alive (Group B1), while 34 patients developed additional morbidities (Group B2) and 11 patients (18%) of Group A2 died. Mean estimated plasma ccf-mtDNA levels were significantly higher in Group B than in Group A, but patients of Group B1 had significantly lower ccf-mtDNA levels than patients of Group B2. Patients developed adult respiratory distress syndrome (ARDS) had significantly higher ccf-mtDNA levels than patients developed sepsis or acute myocardial infarction (AMI) with significantly higher levels in patients developed sepsis. Estimated plasma ccf-mtDNA levels at time of admission showed positive significant correlation with morbidity rate. Odds ratio (OR) for relative risk for development of additional morbidities in patients who had a high plasma ccf-mtDNA level was 26.35. At admission plasma ccf-mtDNA levels in survivors were significantly lower than in non-survivors, and OR was 4.0806. High plasma ccf-mtDNA showed high sensitivity as predictor for ICU mortality.

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*Conclusion:* High at ED admission plasma ccf-mtDNA levels could predict development of additional morbidities during ICU stay of acute trauma patients and showed high sensitivity for prediction of their survival. Very high plasma ccf-mtDNA levels could predict patients liable to develop ARDS.



## 1. Introduction

Plasma DNA can be defined as fragments of DNA that are detectable in the extracellular fluid. There are two types of DNA present in the circulation, circulating cell free DNA (ccfDNA) present in the plasma, which includes DNA packed into nucleosomes from apoptotic cells or DNA associated with circulating lymphocytes which is considered as a minor component [1].

Concomitantly, fragments of mitochondrial DNA are released into the bloodstream. In healthy individuals, the concentration of circulating cell free mitochondrial DNA (ccf-mtDNA) is low, as dead cells are efficiently removed from the circulation by phagocytes. Circulating cfDNA has a short half-life and is removed mainly by the liver. Excessive accumulation of ccf-mtDNA in the plasma may result from the release of DNA caused by massive cell death, inefficient removal of dying cells or a combination of both [2].

Circulating cf-mtDNA fragments induce direct activation of polymorphonuclear neutrophils via Toll-like receptor-9 and formyl-peptide receptor-1 and a SIRS phenotype. The presumption of this reaction is based on the homology of mtDNA with pathogen associated molecular patterns recognized as foreign by the body [3].

Concentrations of plasma ccf-mtDNA are increased in various diseases and have shown some prognostic value in many patient groups, including critically ill patients. Pathophysiological processes behind the need for mechanical ventilation and the treatment itself could raise plasma levels of ccf-mtDNA [4].

Worldwide, injuries resulted in 4.8 million deaths in 2013, an increase of 11.0% since 1990 [5]. Most of these deaths occurred in low- and middle-income countries. World Health Organization estimated that injury is responsible for more deaths than HIV, malaria, and tuberculosis combined [6].

Plasma ccfDNA is a potential marker for trauma prognosis especially death, after severe injury. Circulating cfDNA seems to be connected with injury type and mode, open wounds and surgical operations, which may be the primary reasons for plasma cfDNA increase [7].

## 2. Aim of the study

We assessed whether at admission plasma circulating cell free mitochondrial DNA (ccf-mtDNA) is related to injury severity and can predict morbidity and mortality in acute trauma patients admitted to ICU.

## 3. Patients and methods

The current study was conducted at Department of Anesthesia and ICU at Kasr Al-Ini University Hospital since January 2013 till May 2014. After approval of the study protocol by the Local Ethical Committee and obtaining written fully informed consent from the nearest relative attending with the patient, all patients with solitary or multiple trauma were enrolled in the study. The study also included 10 sex- and age-matched healthy volunteers to give blood samples as control for plasma ccf-mtDNA levels (Group A).

Collected data included age, gender, time lapsed since trauma inflection and arrival to Emergency Department (ED), and injury related data including site, multiplicity, type and cause of trauma. All patients received first aid and resuscitative measures and were clinically evaluated using the Abbreviated Injury Scale (AIS) which is an anatomical injury scoring system on a scale of 1–6 grades: 1 indicated minor, 2 indicated moderate, 3 indicated serious, 4 indicated severe, 5 indicated critical and 6 indicated non-survivable injury. Then, each injury was allocated to one of six body regions including Head, Face, Chest, Abdomen, Extremities and Pelvis, External and the highest AIS score in each body region is used. The 3 most severely injured body regions have their score squared and added together to produce the Injury Severity Scale (ISS) score. The ISS provides an overall score for patients with multiple injuries taking values from 0 to 75. If an injury is assigned an AIS of 6 (unsurvivable injury), the ISS score is automatically assigned to score of 75. The ISS score is virtually the only anatomical scoring system in use and correlates linearly with mortality, morbidity, hospital stay and other measures of severity [8].

Only patients required admission to ICU either preoperatively or postoperatively were included in the study as Group B. Patients who passed smooth ICU stay without development of additional morbidities and were discharged alive were grouped as Group B1 and patients who developed additional morbidities and/or mortality during their ICU stay were grouped as Group B2.

## 4. Blood sampling and processing

At time of arrival to ED, prior to any manipulations or applying lines of treatment, 5 ml peripheral blood sample was obtained under complete aseptic conditions into EDTA-containing tubes. Blood samples were centrifuged at 1600*g* for 10 min, and plasma was removed, with great care taken not to disturb the cell pellet, placed in clean plain polypropylene tubes, and stored at −80 °C till be processed. Blood samples of the healthy controls were also collected and processed in the same way.

## 5. Preparation and quantification of plasma DNA

### 5.1. DNA isolation from plasma

Circulating cell-free mitochondrial DNA (ccf-mtDNA) was extracted from plasma by use of a QIAamp Blood Kit (Qiagen

GmbH) according to manufacturer's instruction [9]. Samples were thawed on ice and were then mixed briefly by vortex, and then 100 μl of plasma was mixed with 100 μl of PBS, followed by brief vortex. Diluted plasma was centrifuged at 700*g* at 4 °C for 5 min, and the supernatant (190 μl) was carefully pipetted taking care not to touch any pellets or the bottom of the tubes with pipette tips. The obtained supernatant was further centrifuged at 18,000*g* at 4 °C for 15 min, and the resulting supernatant (170 μl) was obtained taking care of supernatant contamination because contamination of cells, cell debris, or pellets into supernatant might lead to a significant change of the results.

The obtained supernatant was processed for DNA isolation. In brief, samples were incubated with lysis buffer (included in the kit) and proteinase K at 56 °C for 15 min. At the final step of DNA isolation, DNA was eluted in 200 μl of elution buffer (included in the kit). For the quantitative real-time polymerase chain reaction (qPCR) assay, the DNA solution was further diluted 10 times with nuclease-free deionized, distilled $H_2O$.

### 5.2. Primers and qPCR

DNA level in diluted samples was measured by SYBR Green dye-based qPCR assay using a PRISM 7300 sequence detection system (Applied Biosystems). The primer sequences were as follows: human NADH dehydrogenase 1 gene (mtDNA): forward 5′-ATA CCC ATG GCC AAC CTC CT-3′, reverse 5′-GGG CCT TTG CGT AGT TGT AT-3′ [9]. Bacterial 16S ribosomal RNA: forward 5′-CGT CAG CTC GTG TTG TGA AA-3′, reverse 5′-GGC AGT CTC CTT GAG TTC C-3′ [3]. Plasmid DNA with complementary DNA sequences for human mtDNA was obtained from ORIGENE (SC101172), and plasmid DNA with complementary DNA sequences for human nuclear DNA was obtained from Sino Biological. DNA solutions were diluted in 10-fold serial dilutions and used as standards. Thermal profile for detecting mtDNA was carried out as follows: an initiation step for 2 min at 50 °C is followed by a first denaturation step for 10 min at 95 °C and a further step consisting of 40 cycles for 15 s at 95 °C and for 1 min at 60 °C. All samples were analyzed in duplicate, and a no-template control was included in every analysis. mtDNA levels in all of the plasma analyses were expressed in copies/μl of plasma [10].

### 5.3. Study outcome

**Primary outcome:** Primary outcome is the frequency of survival and additional morbidity rate.

**Secondary outcome:** Secondary outcome is the predictability of at admission plasma ccf-mtDNA level for the prediction of possibility of survival and development of additional morbidity.

### 5.4. Statistical analysis

Sample size was calculated using the standard nomogram proposed by Kraemer and Theimann [11] and a sample size of 30 patients was determined to be sufficient to detect a difference at the 5% significance level and give the trial 60% power [12]. Sample size and power were re-calculated and assured using Power and Sample Size Calculation Software program provided by Department of Biostatistics, Vanderbilt University. Obtained data were presented as mean ± SD, ranges, numbers and ratios. Results were analyzed using One-way ANOVA with post hoc Tukey HSD Test and Chi-square test ($X^2$ test). Possible relationships were investigated using Pearson linear regression. Sensitivity and specificity of ccf-mtDNA as predictor for patients' outcome (survivors or non-survivors) were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC). Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. $P$ value $< 0.05$ was considered statistically significant.

## 6. Results

The study included 92 trauma patients admitted to ED; details of patients' demographic, clinical and trauma-related data are shown in Table 1.

Thirty-one patients were managed conservatively or surgically and were admitted to surgical ward without need for ICU admission and their blood samples were discarded and excluded from the study. Sixty-one patients required admission to ICU (Group B), and 12 patients underwent surgical interference after stabilization of general condition and full investigations and were admitted to surgical ICU for their immediate PO observation. Nine patients showed hemodynamic deterioration and underwent emergency surgical interference and were admitted to surgical ICU after surgery. Seven patients underwent surgical interference and were initially admitted to surgical ward but developed complications necessitated their shift to ICU. Fifteen patients had surgical interference and were admitted immediately to ICU for their PO care. Eighteen patients were hemodynamically unstable on arrival to ER with a mean SBP of 73.6 ± 3.1; range: 68–78 mmHg due to hemorrhage secondary to inflected trauma and were admitted immediately to surgical ICU for preoperative preparation and underwent emergency surgery and continued their PO care period at ICU.

Twenty-seven patients (44.3%) passed smooth ICU stay free of morbidity and were discharged alive without wound related complication (Group B1), while 34 patients developed additional morbidities during their ICU stay (Group B2) for a total postoperative morbidity rate of 55.7%. Nineteen patients (31.1%) developed septic morbidities, 13 patients (21.3%) developed ARDS and two patients (3.3%) had acute myocardial infarction (AMI). Fifty patients passed their additional morbidities successfully and were discharged from ICU alive for a total survival rate of trauma patients admitted to ICU of 82%. Unfortunately, 11 patients died secondary either to failure to respond to supportive measures for the resultant pathology secondary to inflected trauma and PO decompensation or to their developed additional morbidities. Both patients with AMI, 5 patients with ARDS and 4 patients with sepsis had died for a mortality rate of 18% during ICU stay (Table 2).

Mean ISS was significantly higher in patients developed septic complications and ARDS compared to those passed smooth ICU without additional morbidities (Group B1), but with non-significantly higher ISS score in ARDS patients compared to patients developed sepsis. Mean ISS was

**Table 1** At admission patients' data.

| Data | | | | Findings |
|---|---|---|---|---|
| Age (years) | | Strata | < 20 years | 16 (15.7%) |
| | | | 20–29 | 29 (28.4%) |
| | | | 30–39 | 23 (22.5%) |
| | | | 40–49 | 15 (14.7%) |
| | | | 50–59 | 12 (12.7%) |
| | | | ⩾60 | 6 (5.9%) |
| | | Total | Number | 102 (100%) |
| | | | Mean (±SD) | 35.3 ± 14.1 (15–74) |
| Gender | | Males | | 73 (71.6%) |
| | | Females | | 29 (28.4%) |
| Time lapsed between trauma (minutes) | | Strata | < 60 | 78 (76.5%) |
| | | | 60–120 | 19 (18.6%) |
| | | | > 120 | 5 (4.9%) |
| | | Total | Mean (±SD) | 56.8 ± 27.7 (20–140) |
| Site and multiplicity of injury | | Solitary injury | Head and neck | 9 (8.8%) |
| | | | Chest | 18 (17.6%) |
| | | | Abdomen | 32 (31.4%) |
| | | | Extremities | 14 (13.7%) |
| | | Multiple body trauma | | 29 (28.5%) |
| Type and cause of trauma | Penetrating ($n$ = 61; 59.8%) | Bullet | Single | 5 (4.9%) |
| | | | Multiple | 4 (3.9%) |
| | | Gunshots | | 7 (6.9%) |
| | | Knives | Single | 4 (3.9%) |
| | | | Multiple | 6 (5.8%) |
| | | Car accident | | 26 (25.4%) |
| | | Sharp objects | | 9 (8.8%) |
| | Blunt ($n$ = 41; 40.2%) | Car accident | | 19 (18.6%) |
| | | Fall from height | | 12 (11.8%) |
| | | Crush injury | | 10 (9.8%) |
| Mortality at ED according to multiplicity of truma | | Solitary body trauma | | 6 (5.8%) |
| | | Multiple body trauma | | 4 (3.9%) |
| | | Total | | 10 (9.8%) |

Data are shown as numbers and mean ± SD; percentages and ranges are in parenthesis.

**Table 2** Outcome of patients admitted to ICU.

| Group | | Survived | Died | Total |
|---|---|---|---|---|
| Group A1 (Free of additional morbidities) | | 27 (44.3%) | 0 | 27 (44.3%) |
| Group A2 (Developed additional morbidities) | Sepsis | 15 (24.5%) | 4 (6.6%) | 19 (31.1%) |
| | ARDS | 8 (13.1%) | 5 (8.2%) | 13 (21.3%) |
| | AMI | 0 | 2 (3.3%) | 2 (3.3%) |
| Total | | 50 (82%) | 11 (18%) | 61 (100%) |

Data are presented as numbers; percentages are in parenthesis; ARDS: Adult respiratory distress syndrome; AMI: Acute myocardial infarction.

non-significantly higher in patients developed AMI compared to patients of group B1, but was non-significantly lower compared to those developed sepsis or ARDS. Mean plasma ccf-mtDNA levels of patients of group B were significantly higher compared to control level (Group A). However, patients of Group B1 had significantly lower plasma ccf-mtDNA compared to patients of Group B2. Patients developed ARDS had significantly higher plasma ccf-mtDNA levels compared to patients developed sepsis or AMI with significantly higher levels in patients developed sepsis compared to patients developed AMI (Table 3).

At admission plasma ccf-mtDNA levels showed positive significant correlation with the frequency of additional morbidities development ($r$ = 0.538, $p$ = 0.0006). The odds ratio for relative risk for development of additional morbidities in patients had high plasma ccf-mtDNA level was 26.3455 (95% CI is 1.4774–469.791; $Z$ = 2.226, $p$ = 0.026) (Fig. 1). Mean at admission plasma ccf-mtDNA levels in survivors (4011.6 ± 3885 copies/μl) was significantly ($p$ = 0.045) lower compared to non-survivors (11040.9 ± 9116 copies/μl) (Fig. 2). The odds ratio for relative risk for death of patients had high plasma ccf-mtDNA level was 4.0806 (95% CI is

**Table 3** Mean at admission plasma ccf-mtDNA levels and ISS scores of enrolled patients' categorized according to outcome.

| Group | | Plasma ccf-mtDNA | | ISS | |
|---|---|---|---|---|---|
| | | Level | *P* value | Level | *P* value |
| Control (Group A; $n = 10$) | | 879 ± 227.6 | | 0 | |
| Group B1 (Free of additional morbidities; $n = 27$) | | 1428.9 ± 463.2 | $P_1 = 0.0005$ | 15.9 ± 4.7 | |
| Group B2 (Developed additional morbidities) | Sepsis ($n = 19$) | 4515.8 ± 2040.8 | $P_1 = 0.0006$<br>$P_2 = 0.00006$ | 21 ± 7.6 | $P_2 = 0.015$ |
| | ARDS ($n = 13$) | 14892.3 ± 8015.4 | $P_1 = 0.0008$<br>$P_2 = 0.0009$<br>$P_3 = 0.004$ | 24.1 ± 2.8 | $P_2 = 0.001$<br>$P_3 > 0.05$ |
| | AMI ($n = 2$) | 2025 ± 106.1 | $P_1 = 0.0007$<br>$P_2 = 0.0009$<br>$P_3 = 0.001$<br>$P_4 = 0.001$ | 20.5 ± 4.9 | $P_2 > 0.05$<br>$P_3 > 0.05$<br>$P_4 > 0.05$ |
| Total | | 5279.2 ± 6436.1 | $P_1 = 0.0003$ | 19.4 ± 6.3 | |

Data are presented as mean ± SD; ARDS: Adult respiratory distress syndrome; AMI: Acute myocardial infarction; $P_1$: significance versus control levels; $P_2$: significance versus levels of patients of group B1; $P_3$: significance versus levels of patients developed sepsis; $P_4$: significance versus levels of patients developed ARDS.

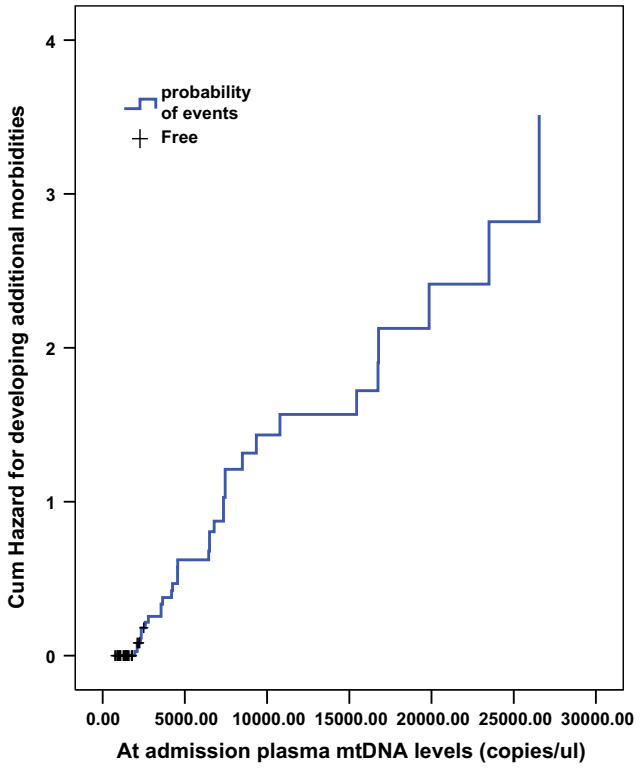


**Figure 1** Cumulative hazard for development of additional morbidities in patients had at admission high mtDNA level.

0.2588–64.34; $Z = 0.999$, $p > 0.05$) (Fig. 3). ROC curve analysis showed significantly small area under curve for predictability of at admission plasma ccf-mtDNA for survival indicating high sensitivity of high plasma levels as a predictor for ICU mortality (Fig. 4).

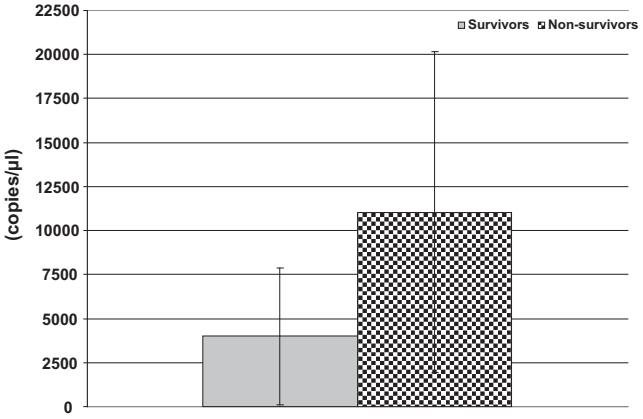


**Figure 2** Mean (±SD) plasma mtDNA levels estimated in survivors compared to non-survivors.

## 7. Discussion

The current study detected a significantly higher plasma ccf-mtDNA levels in acute trauma patients admitted to ICU compared to levels estimated in control healthy volunteers. Patients developed additional morbidities during ICU stay were found to have significantly higher levels compared to both levels of controls and patients who did not develop additional morbidities. Moreover, estimated ccf-mtDNA levels were significantly higher in patients developed ARDS than in patients who developed septic morbidities and acute myocardial infarction (AMI) with significantly higher levels in patients developed septic morbidities than in patients developed AMI.

These findings go in hand with Simmons et al. [13] who reported that patients with SIRS had significantly increased ccf-mtDNA levels in all sequences examined and in patients who developed multiple-organ dysfunction (MOD) than patients who did not develop MOD. Gu et al. [14] also found median plasma ccf-mtDNA was significantly higher in trauma

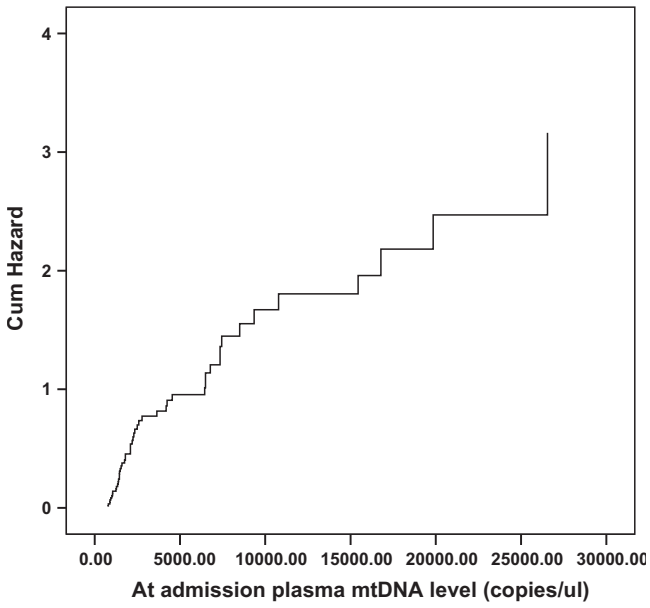


**Figure 3** Cumulative hazard for mortality in patients had at admission high mtDNA level.

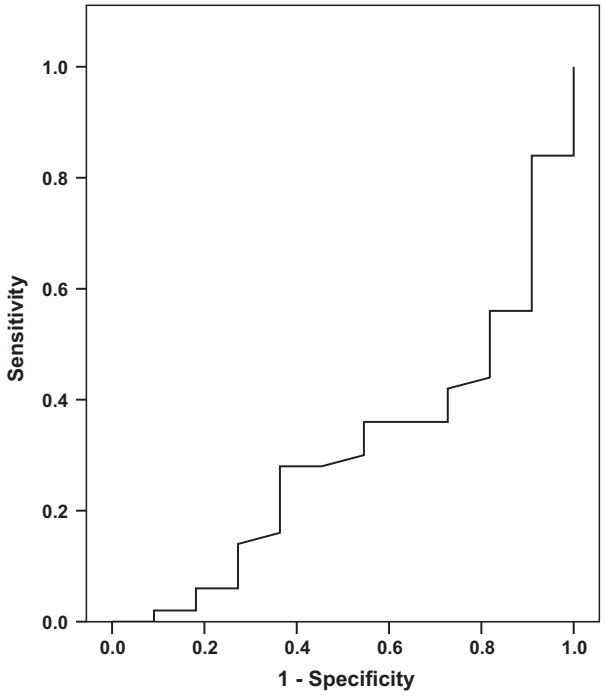


**Figure 4** ROC curve analysis for predictability of at admission high mtDNA level for mortality of enrolled patients.

patients than in healthy controls. Recently, in 2016, Clementi et al. [15] detected significantly higher levels of cfDNA in septic patients than controls and in septic patients who developed acute kidney injury (AKI) requiring renal replacement therapy than in patients who did not develop AKI.

Relative risk (RR) for patients had at admission high plasma ccf-mtDNA to develop additional morbidities was high and odd ratio (OR) was significant with curve showing condensation of free patients in narrow low range indicating its predictability for future morbidities as specific predictor. Concerning mortality, ROC curve analysis showed high sensitivity to predict mortality with narrow AUC that was significantly smaller compared to the null hypothesis that AUC = 0.5 is nonsense.

In line with the predictability of high plasma ccf-mtDNA for morbidity and mortality, Kung et al. [16] concluded that plasma ccf-mtDNA has potential use for predicting outcome in septic patients arriving at ED and on admission plasma ccf-mtDNA level is a more powerful predictor for fatality of severe septic patients than on admission lactate concentration or SOFA scores. Yamanouchi et al. [17] reported that in trauma patients, day-1 concentrations of ccf-mtDNA significantly correlated with the maximal levels of creatinine phosphokinase and were significantly higher in non-survivors compared with survivors of trauma. Simmons et al. [13] found patients with above-median ccf-mtDNA levels had a significantly elevated RR for mortality and concluded that plasma ccf-mtDNA is associated with the evolution of SIRS, MOD, and mortality in severely injured human subjects.

Gu et al. [14] also reported that regression analysis revealed that the plasma ccf-mtDNA was an independent predictor for post-traumatic SIRS and ROC analysis demonstrated that a high plasma ccf-mtDNA level predicted post-traumatic SIRS with sensitivity and specificity of 67% and 76%, respectively. Nakahira et al. [18] assessed analyses of ccf-mtDNA levels in two prospective observational cohort studies of ICU patients and concluded that increased ccf-mtDNA levels are associated with ICU mortality and improved risk prediction in ICU patients and it could serve as a viable plasma biomarker for ICU patients.

Yang et al. [19] reported that the concentration of ccf-mtDNA in blood was found to be a prognostic indicator for ICU mortality in patients with severe sepsis. Recently, in 2016, Clementi et al. [15] documented that ccf-mtDNA can be considered as a good prognostic marker of clinical outcome in septic patients with its increased levels are associated with poor outcome and correlate with caspase-3, interleukin 6 and 18 levels.

Multiple studies tried to explore the underlying mechanisms for development of additional morbidities in trauma patients with high plasma ccf-mtDNA; Zhang et al. [3] reported that injury releases mitochondrial damage-associated molecular patterns (DAMPs) including ccf-mtDNA into the circulation; it activates human poly-morphonuclear neutrophils (PMN) and promotes PMN calcium ion flux and phosphorylation of mitogen-activated protein kinases, thus leading to PMN migration and degranulation in vitro and in vivo and elicit neutrophil-mediated organ injury, thus creating a sepsis-like state and concluded that the release of such mitochondrial 'enemies within' by cellular injury is a key link between trauma, inflammation and SIRS.

Gomez et al. [20] found that in trauma patients who carry the C-allele, T4216C polymorphism in the NADH dehydrogenase 1 gene was less able to generate the cellular energy necessary to mount an effective immune response relative to carriers of the T-allele and showed significantly increased risk for sepsis complicated by MOD or septic shock as well as death. Lorente et al. [21] found that septic patients with ccf-mtDNA haplogroup JT showed increased 30-day and 6-month survival and concluded that this finding may be due to single nucleotide

polymorphism defining the whole haplogroup JT and not separately for J or T sub-haplogroups. Fernández-Ruiz et al. [22] found that exposure of monocyte to ccf-mtDNA induces endotoxin tolerance which is characterized by decreased production of cytokines in response to pro-inflammatory stimuli.

In the current study, blood samples were obtained at time of ED admission and prior to manipulation; in line with such timing Kung et al. [16] found on admission median plasma ccf-mtDNA and nuclear DNA levels were significantly higher in severe septic patients compared to controls and Yamanouchi et al. [17] also reported that in patients with trauma, concentrations of ccf-mtDNA peaked on the day-1 of admission. Moreover, Ren et al. [7] reported that positive plasma ccf-mtDNA samples were higher 1–6 h after injury than 24–48 h and 60–90 h.

## 8. Conclusion

High at ED admission plasma ccf-mtDNA levels could predict development of additional morbidities during ICU stay of acute trauma patients and showed high sensitivity for prediction of their survival. Very high plasma ccf-mtDNA levels could predict patients liable to develop ARDS.

## 9. Limitation of the study

One of limitation points was inclusion of trauma patients only; the obtained results needed to be adjusted for patients admitted to ICU, irrespective of diagnosis. Another point was the cost of PCR estimation of plasma ccf-mtDNA to advise its wide spread applicability.

## Conflict of interest

No conflict of interest.

## References

[1] Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001;61:1659–65.
[2] Zeerleder S, Zwart B, Wuillemin WA, Aarden LA, Groeneveld AB, Caliezi C, et al. Elevated nucleosome levels in systemic inflammation and sepsis. Crit Care Med 2003;31(7):1947–51.
[3] Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. Nature 2010;464(4):104–8.
[4] Okkonen M, Lakkisto P, Korhonen AM, Parviai-nen I, Reinikainen M, Varpula T, et al. FINNALI study group: plasma cell-free DNA in patients needing mechanical ventilation. Crit Care 2011;15(4):R196.
[5] Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2015;385(9963):117–71.
[6] Gosselin RA, Spiegel DA, Coughlin R, Zirkle LG. Injuries: the neglected burden in developing countries. Bull World Health Organ 2009;87(4), 24–24a.
[7] Ren B, Liu F, Xu F, He J, Zhu H, Zou G. Is plasma cell-free DNA really a useful marker for diagnosis and treatment of trauma patients? Clin Chim Acta 2013;424:109–13.
[8] Copes WS, Champion HR, Sacco WJ, Lawnick MM, Keast SL, Bain LW. The injury severity score revisited. J Trauma 1988;28 (1):69–77.
[9] Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 2011;12:222–30.
[10] Nga TV, Karkey A, Dongol S, Thuy HN, Dunstan S, et al. The sensitivity of real-time PCR amplification targeting invasive Salmonella serovars in biological specimens. BMC Infect Dis 2010;10:125.
[11] Kraemer HC, Theimann S. How many subjects? Statistical power analysis in research. Newbury Park (CA): Sage; 1987.
[12] Murphy KR, Myors B. Statistical power analysis: a simple and general model for traditional and modern hypothesis tests. 2nd ed. Lawrence Erlbaum Associates Inc.; 2003.
[13] Simmons JD, Lee YL, Mulekar S, Kuck JL, Brevard SB, Gonzalez RP, et al. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. Ann Surg 2013;258(4):591–8.
[14] Gu X, Yao Y, Wu G, Lv T, Luo L, Song Y. The plasma mitochondrial DNA is an independent predictor for post-traumatic systemic inflammatory response syndrome. PLoS ONE 2013;8(8):e72834.
[15] Clementi A, Virzì GM, Brocca A, Pastori S, de Cal M, Marcante S, et al. The role of cell-free plasma DNA in critically Ill patients with sepsis. Blood Purif 2016;41(1–3):34–40.
[16] Kung CT, Hsiao SY, Tsai TC, Su CM, Chang WN, Huang CR, et al. Plasma nuclear and mitochondrial DNA levels as predictors of outcome in severe sepsis patients in the emergency room. J Transl Med 2012;10:130.
[17] Yamanouchi S, Kudo D, Yamada M, Miyagawa N, Furukawa H, Kushimoto S. Plasma mitochondrial DNA levels in patients with trauma and severe sepsis: time course and the association with clinical status. J Crit Care 2013;28(6):1027–31.
[18] Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. PLoS Med 2013;10(12):e1001577.
[19] Yang J, Selvaganapathy PR, Gould TJ, Dwivedi DJ, Liu D, Fox-Robichaud AE, et al. A microfluidic device for rapid quantification of cell-free DNA in patients with severe sepsis. Lab Chip 2015;15(19):3925–33.
[20] Gomez R, O'Keeffe T, Chang LY, Huebinger RM, Minei JP, Barber RC. Association of mitochondrial allele 4216C with increased risk for complicated sepsis and death after traumatic injury. J Trauma 2009;66(3):850–8.
[21] Lorente L, Iceta R, Martín MM, López-Gallardo E, Solé-Violán J, Blanquer J, et al. Severe septic patients with mitochondrial DNA haplogroup JT show higher survival rates: a prospective, multicenter, observational study. PLoS ONE 2013;8(9):e73320.
[22] Fernández-Ruiz I, Arnalich F, Cubillos-Zapata C, Hernández-Jiménez E, Moreno-González R, Toledano V, et al. Mitochondrial DAMPs induce endotoxin tolerance in human monocytes: an observation in patients with myocardial infarction. PLoS ONE 2014;9(5):e95073.