

Plasma nuclear and mitochondrial DNA levels in acute myocardial infarction patients

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Objective Plasma nuclear and mitochondrial DNA (mtDNA) levels are altered in many diseases. However, it is not known whether they are also altered in acute myocardial infarction (AMI). In the present study, we examined plasma nuclear and mtDNA levels in the patients with AMI before and after a percutaneous coronary intervention (PCI) to explore their potential as biomarkers.

Methods and results Plasma nuclear and mtDNA levels were measured by quantitative PCR in 25 AMI patients, 25 non-myocardial infarction (MI) control participants (with MI risk), and 20 healthy individuals during the study period. The concentrations of nuclear and mtDNA were significantly higher in the AMI group on hospital day 1 than that in the non-MI controls (nuclear: 0.4948 ± 0.0830 vs. 0.2047 ± 0.0222 ng/ μ l, $P < 0.05$; mitochondrial: 3.754 ± 0.384 vs. 1.851 ± 0.3483 ng/ μ l, $P < 0.05$) and healthy individuals (nuclear: 0.4948 ± 0.0830 vs. 0.1683 ± 0.0254 ng/ μ l, $P = 0.001$; mitochondrial: 3.754 ± 0.384 vs. 0.1517 ± 0.0924 ng/ μ l, $P < 0.05$) and decreased shortly after PCI.

Introduction

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide [1]. Partial or complete epicardial coronary artery occlusion from plaques vulnerable to rupture or erosion is the most common cause of MI [2]. MI may be a minor event in a lifelong chronic disease and may even go undetected, but it may also be a major catastrophic event leading to sudden death or severe hemodynamic deterioration. The term MI reflects cell death of cardiomyocyte caused by ischemia, which is the result of a perfusion imbalance between supply and demand. Because of tissue damage and necrosis of cardiac cells, danger signals, such as extracellular matrix breakdown products, mitochondrial DNA (mtDNA), heat shock proteins, and high mobility box 1, are released [3].

Over the years, the use of more specific and sensitive biomarkers of myocardial necrosis has improved the detection of MI. For example, lactate dehydrogenase was shown to be better than glutamine-oxaloacetic transaminase and replaced it in the diagnosis of MI, and later,

Conclusion Both plasma nuclear and mtDNA levels are elevated in AMI patients, but return to normal levels immediately after PCI, suggesting that they are potentially novel biomarkers for AMI. *Coron Artery Dis* 26:296–300 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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creatinase (CK) and the MB fraction of CK, that is, CKMB activity and CKMB mass were used instead for the diagnosis [4]. Nevertheless, more specific and sensitive biomarkers to detect MI are required.

Mitochondria are double-membrane organelles in the cytoplasm of eukaryotic cells and contain their own DNA. They carry out oxidative phosphorylation to produce ATP that is required for many cellular activities as energy [5]. Recent studies have shown that mtDNA is released as damage-associated molecular patterns into circulation after aseptic trauma and its levels correlate with the incidence of distant organ failure and death [6–8]. mtDNA that escapes from autophagy cell autonomously has been shown to act through toll-like receptor 9 to induce inflammation and cardiomyocyte injury [9].

mtDNA could be part of plasma cell-free DNA, which includes both mtDNA and nuclear DNA [10]. A recent study shows that plasma nuclear DNA concentrations increase proportionately to the complications arising from acute coronary syndrome [11]. Moreover, another study has shown that acute myocardial infarction (AMI) can lead to an increase in circulating mtDNA [12]. However, the changes in circulating nuclear and mtDNA levels have not been examined simultaneously in AMI.

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We hypothesized that nuclear and mtDNA contents are increased after AMI and decreased by a percutaneous coronary intervention (PCI). In a clinical setting, mtDNA or nuclear DNA may serve as a prognostic and/or a diagnostic biomarker in AMI.

Methods

Ethics statement

The present study protocol was approved by Jinling Hospital's Institutional Review Committee on Human Research. All experimental procedures and written informed consent obtained from all donors were reviewed and approved by the Local Ethics Committee.

Enrollment of patients and normal controls

A total of 25 AMI patients were enrolled from Jinling Hospital between January 2014 and April 2014. AMI was diagnosed according to the European Society of Cardiology (ESC), the American College of Cardiology, and American Heart Association (ACC/AHA) redefined guidelines. Exclusion criteria were as follows: surgery, trauma, previous transmural infarction, cardiogenic shock, severe liver disease, renal failure, underlying neoplasm, hematologic disorders that affect platelet count or function, and fever or infectious conditions upon study entry. Twenty-five control participants without acute coronary syndrome, whose routine coronary angiography was negative, served as non-MI controls. Twenty healthy volunteers served as an internal control. This study was approved by the local ethics committees. All participants signed an informed consent.

Plasma preparation

In the study, 5 ml of whole blood samples were drawn at hospital days 1 (within 8 h of admission) and 3 (2 days after PCI), transferred into EDTA-coated blood collection tubes, and processed within 2 h after venipuncture. Briefly, whole blood was centrifuged at 500 *g* at room temperature for 10 min, and the supernatant was transferred to a fresh tube and centrifuged at 700 *g* at 4°C for 5 min. Then, the supernatant (240 μ l) obtained from the whole blood (5 ml) was collected carefully using a pipette without touching the pellet or the bottom of the tube. The supernatant obtained was further centrifuged at 15 000 *g* at 4°C for 10 min, and the resulting supernatant (200 μ l) was collected carefully. The plasma samples were stored at -80°C and were used for DNA isolation within 4 months of storage.

DNA isolation from plasma

Plasma DNA was isolated from plasma using the QIAamp DNA Blood Mini Kit (#51104; Qiagen, Valencia, California, USA) following the manufacturer's manual [13]. In brief, samples were thawed on ice and were then mixed briefly by vortex. Then, we incubated the plasma samples with lysis buffer and proteinase K at 56°C for 10 min. At the final step of isolation, DNA was eluted

with 150 μ l of nuclease-free deionized and distilled H₂O, followed by a quantitative real-time PCR assay.

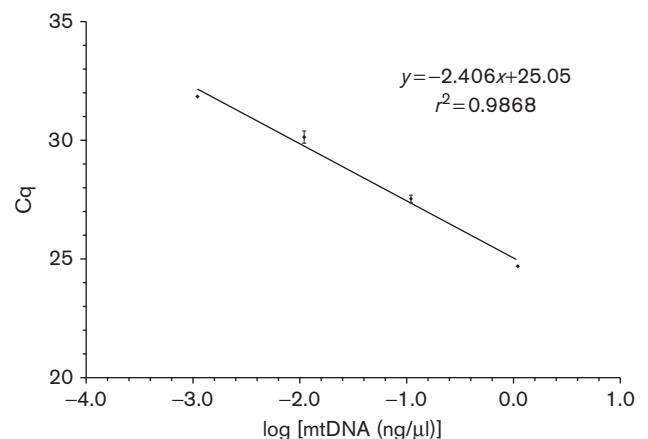
Plasma DNA quantification by qPCR

mtDNA and nuclear DNA were quantified by real-time PCR using the Lightcycler 96 sequence detection system (Roche, Mannheim, Germany) with the following primers: human NADH dehydrogenase 1 gene (mtDNA): forward 5'-ATACCCATGGCCAACCTCCT-3', reverse 5'-GGGCCTTTGCGTAGTTGTAT-3' [6,14]; human b-globin (nuclear DNA): forward 5'-GTGCACCTGACTCCTGAGGAGA-3', reverse 5'-CCTTGATACCAACCTGCCAG-3' [15]. The thermal profile for mtDNA quantitative real-time PCR was as follows: denaturation at 95°C for 10 min, followed by 40 cycles of 10 s at 95°C, 10 s at 58°C, and 10 s at 72°C. In each application, samples were analyzed in duplicate and the mean was used in the subsequent analysis. The concentration of the standards was quantified spectrophotometrically (Nano Drop 2000; Thermo Fischer, Wilmington, Delaware, USA). The standard curve is shown in Fig. 1. The unknown samples were compared with the standard curve. Plasma DNA concentrations were expressed as ng/ μ l.

Statistical analysis

Statistical analyses were carried out using the statistical package for social sciences (SPSS Inc., Chicago, Illinois, USA). Results are shown as the mean \pm SEM. Paired data were evaluated using a Student's *t*-test. A one-way analysis of variance with the Bonferroni post-hoc test was used for multiple comparisons. The χ^2 -test was used to assess the association between two categorical variables. *P* value less than 0.05 was considered statistically significant.

Fig. 1



Standard curve for mtDNA quantitative real-time PCR. The equation of the standard curve in the assay was $y = -2.406x + 25.05$. The linear regression line had an r^2 value of 0.9868. mtDNA, mitochondrial DNA.

Results

The AMI and control groups were similar in baseline clinical characteristics

The baseline characteristics of the two groups are listed in Table 1. Twenty-five adults (four women and 21 men, aged 44–81 years) with AMI were evaluated. The mean age of the AMI patients was 59.3 ± 13.4 years and that of the controls was 64.5 ± 12.0 years ($P=0.165$). According to the ECG criteria, 14 patients showed signs of inferior/posterior wall infarction and 11 patients showed signs of anterior wall infarction. There were no significant differences in other vascular risk factors, including hypertension, diabetes mellitus, dyslipidemia, and smoking status, between the two groups.

Plasma nuclear and mtDNA in AMI and control groups

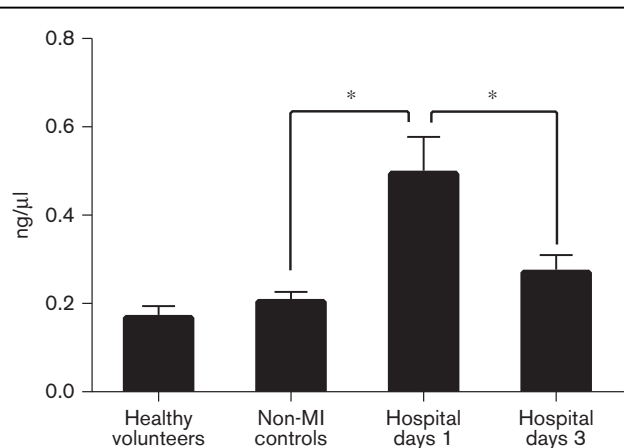
The levels of plasma nuclear and mtDNA in patients with AMI, non-MI controls, and healthy volunteers are shown in Figs 2 and 3. The concentrations of nuclear and mtDNA were significantly higher in the AMI group on hospital day 1 than that in the non-MI controls (nuclear: 0.4948 ± 0.0830 vs. 0.2047 ± 0.0222 ng/ μ l, $P < 0.05$; mitochondrial: 3.754 ± 0.384 vs. 1.851 ± 0.3483 ng/ μ l, $P < 0.05$) and healthy individuals (nuclear: 0.4948 ± 0.0830 vs. 0.1683 ± 0.0254 ng/ μ l, $P = 0.001$; mitochondrial: 3.754 ± 0.384 vs. 0.1517 ± 0.0924 ng/ μ l, $P < 0.05$). There was no significant difference in the concentration of plasma nuclear DNA between the non-MI controls and the healthy individuals (0.2047 ± 0.0222 vs. 0.1683 ± 0.0254 ng/ μ l, $P > 0.05$). Plasma levels of nuclear DNA were 0.4948 ± 0.0830 ng/ μ l in the patients with AMI on hospital day 1 and 0.2709 ± 0.0386 ng/ μ l on hospital day 3 ($P < 0.05$). Levels of plasma mtDNA were 3.754 ± 0.384 ng/ μ l in the patients with AMI on hospital day 1 and 2.112 ± 0.213 ng/ μ l on hospital day 3 ($P < 0.05$).

Table 1 Characteristics of the study patients with and without AMI

Characteristics	Patients with AMI (n = 25)	Patients without AMI (n = 25)	P value
Age (years)	59.3 ± 13.4	64.5 ± 12.0	0.165
Male/female (n/n)	21/4	18/7	0.496
Current smoking [n (%)]	5 (20)	7 (28)	0.742
DM ₁ [n (%)]	4 (16)	6 (24)	0.725
Hypertension [n (%)]	17 (68)	13 (52)	0.387
Hyperlipidemia [n (%)]	2 (8)	3 (12)	1.000
SBP ₂ (mmHg)	133 ± 22	136 ± 18	0.572
DBP ₃ (mmHg)	80 ± 15	76 ± 7	0.298
TC ₄ (mmol/l)	4.26 ± 0.91	3.82 ± 0.80	0.084
TG ₅ (mmol/l)	1.32 ± 0.45	1.73 ± 0.91	0.057
HDL ₆ (mmol/l)	1.00 ± 0.24	0.97 ± 0.19	0.709
LDL ₇ (mg/dl)	2.73 ± 0.70	2.36 ± 0.74	0.082
WBC ₈ /nl	9.92 ± 0.82	9.17 ± 1.50	0.143
Creatinine (μ mol/l)	66.96 ± 16.21	60.08 ± 17.06	0.150
Urea (mmol/l)	5.71 ± 1.61	5.96 ± 1.80	0.599

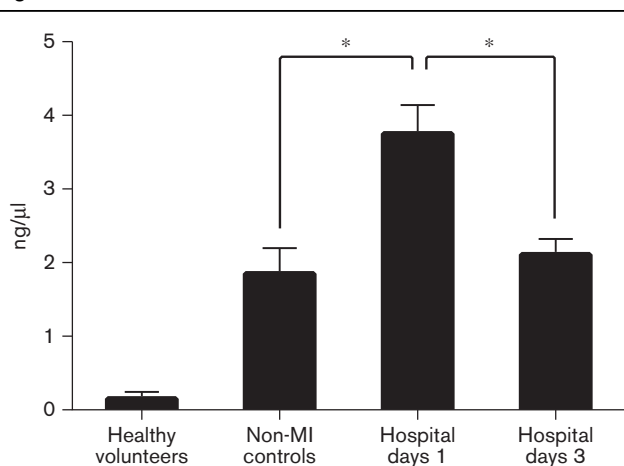
AMI, acute myocardial infarction; DBP, diastolic blood pressure; DM, diabetes mellitus; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WBC, white blood cell count.

Fig. 2



Concentrations of plasma nuclear DNA on different days in AMI patients and control participants. Each bar was expressed as mean \pm SEM. * $P < 0.05$. AMI, acute myocardial infarction; MI, myocardial infarction.

Fig. 3



Concentrations of plasma mitochondrial DNA on different days in AMI patients and control participants. Each bar was expressed as mean \pm SEM. * $P < 0.05$. AMI, acute myocardial infarction; MI, myocardial infarction.

Discussion

We evaluated circulating nuclear and mtDNA levels in the patients with AMI. This is the first study that has shown marked increases in circulating nuclear and mtDNA levels in the patients with AMI compared with non-AMI patients and decreases after PCI. Our work has confirmed and extended previous studies showing that a high level of mtDNA or cell-free DNA may be accompanied by myocardial damage in patients [3,5,10,12, 16–18].

To our knowledge, plasma DNA has been studied in a wide range of human diseases from cancer to diabetes, as well as in development, aging, and exercise [19–24]. The

prognostic and diagnostic utility of plasma DNA has been proven in some critical conditions [25]. Increase in plasma DNA appears to be common in the diseases involving cell death, including infections, cancers with metastasis, hepatitis, irreversible cardiac failure, severe respiratory insufficiency, and thrombophlebitis [26]. The majority of plasma DNA is derived from apoptotic or necrotic cells [24]. The cellular origin of plasma DNA seems to be different in various pathologic conditions, but remains uncertain in most cases. Moreover, mtDNA acts as a damage-associated molecular pattern [27] that can promote molecular processes, leading to inflammatory responses and organ injuries [6,13,28,29]. Inflammation can affect the release of DNA from cells undergoing apoptosis or necrosis, although the nature of this effect may vary depending on the inflammatory stimulus and local cellular events [30]. Furthermore, inflammatory responses and recruitment of neutrophils in AMI are more pronounced than that in chronic heart failure [31]. Therefore, it is possible that mtDNA released from necrotic cells or escaped from autophagy may induce a danger signal, leading to inflammatory responses in AMI.

Currently, cardiac troponin T and troponin I are the best biomarkers for the diagnosis of AMI because of its cardiac specificity and sensitivity [32]. However, measurable amounts of troponin proteins are usually not released from the damaged myocardium before 4–8 h after the onset of symptoms, making an early biomarker-based diagnosis of AMI rather difficult. By contrast, mtDNA arises ahead of time (about 1 h after chest pain) in the plasma [12], potentially making it a novel biomarker for early diagnosis of AMI. However, the specificity and sensitivity of mtDNA in the diagnosis of AMI need to be further investigated.

Our study has several limitations. First, the levels of plasma nuclear and mtDNA may be affected by age and pre-existing diseases [33]. Second, the number of cases in the study was small and the follow-up period was also short. Large-scale prospective studies are warranted to evaluate the diagnostic and prognostic utility of plasma DNA for AMI.

In summary, plasma nuclear and mtDNA levels increase after AMI and peak rapidly. Further studies are essential to show the specificity and sensitivity of mtDNA in the diagnosis of AMI. It is likely that plasma nuclear and mtDNA may serve as biomarkers and should be tested routinely in the future.

Conclusion

Concentrations of plasma nuclear and mtDNA in patients with AMI were significantly higher than those in the non-MI controls and healthy participants and decreased shortly after PCI. Plasma nuclear and mtDNA levels may be a novel biomarker for AMI.

Acknowledgements

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Conflicts of interest

There are no conflicts of interest.

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