



Original Article

Perioperative plasma mitochondrial DNA dynamics and correlation with inflammation during infantile cardiopulmonary bypass



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ABSTRACT

Objective: Numerous studies in animals and humans have demonstrated that inflammatory mediators such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, and IL-8 play a role in cardiopulmonary bypass (CPB), which might affect surgical outcomes. Plasma mitochondrial DNA (mtDNA), a recently discovered pro-inflammatory agent, is released by cells upon insult. This study aimed to detect changes in plasma mtDNA levels at different time points after infantile CPB and explore its potential association with inflammatory mediators.

Methods: In the present study, we analyzed the perioperative plasma mtDNA and inflammatory cytokine levels of 48 infants undergoing ventricular septal defect closure. Blood samples were collected before aortic cross-clamping (T1), at the end of CPB (T2), and 6 h (T3), 12 h (T4), and 24 h (T5) post-CPB. Reverse transcription-polymerase chain reaction and specific enzyme-linked immunosorbent assay were used to quantify the plasma mtDNA and inflammatory cytokines, respectively. Bivariate correlation analysis was used to determine the correlations between plasma mtDNA and inflammatory cytokines.

Results: Plasma mtDNA levels increased at T2 and peaked at T3. Significant positive correlations were found between peak plasma mtDNA (at T3) and several inflammatory biomarkers, including IL-6 (at T3) ($r=0.62$, $P<0.001$), IL-8 (at T2) ($r=0.53$, $P<0.001$), and TNF- α (at T3) ($r=0.61$, $P<0.001$).

Conclusion: Here we report that mtDNA may participate in a systemic inflammatory response to CPB.

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1. Introduction

Outcomes of infantile cardiac surgery have improved dramatically over the past few decades. However, side effects such as low cardiac output and transient end-organ dysfunction followed by the surgery procedures may also occur and even lead to death. Poor outcomes are partly attributed to the use of cardiopulmonary bypass (CPB).¹ Numerous studies in animals and humans have demonstrated that inflammatory mediators such as tumor necrosis factor (TNF)- α , interleukin (IL)-6 and IL-8 play roles in CPB,^{2–4} which can adversely affect surgical outcomes.⁵ Infants are particularly vulnerable to this inflammatory state as a greater percentage of their blood is exposed to the artificial circuit leading

to a more pronounced inflammatory response and more adverse outcome than adults.⁶ Therefore, closer attention is needed to reduce the level of inflammation caused by CPB during infantile individuals. Although many anti-inflammatory agents were investigated to inhibit the inflammatory responses to CPB,^{7,8} further studies are required to reduce the inflammatory responses by fully understanding the unknown mechanism of CPB-induced inflammation.

Human mitochondrial DNA (mtDNA) consists of 16,569 nucleotide bases and is responsible for encoding 13 polypeptides of the electron transport chain, 22 transfer RNAs, and two ribosomal RNAs.⁹ When cells are injured, damaged mitochondria release mtDNA into the circulation, which triggers inflammation.¹⁰ Zhang Q et al. demonstrated that circulatory mtDNA levels were significantly elevated and promoted an inflammatory response in trauma patients.¹¹ Since CPB was a harmful factor to patients, we hypothesized that mtDNA may elevate in circulation and play a key role in the inflammatory response after infantile cardiac surgery with CPB. The present study was designed to detect changes in

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plasma mtDNA levels at different time points after infantile CPB and explore its potential association with inflammatory mediators such as TNF- α , IL-6, and IL-8.

2. Methods

2.1. Study population

This was a prospective, single-center, observational study. Between August 2014 and April 2015, 68 infants with a ventricular septal defect requiring surgical closure were admitted to our institution. Patients with renal dysfunction and a history of autoimmune, systemic inflammatory diseases, or hepatic, lung, or renal disease were excluded. Of the 68 patients screened for participation, the parents of 11 refused to provide consent for participation and nine met at least one exclusion criterion, so 48 patients were ultimately enrolled in this study. Intracardiac ventricular septal defect repair with CPB and postoperative standard care in the cardiac intensive care unit were performed for all patients. The study was approved by the institutional review board of our hospital, and all of the patients' parents or guardians provided written informed consent.

2.2. Blood sample collection

Blood samples were collected into EDTA-coated tubes ahead of aortic cross-clamping (T1), at the end of CPB (T2) as well as 6 h, 12 h and 24 h post-CPB (T3, T4, T5 respectively). The collected blood was centrifuged at 1000 rpm, 4°C for 15 min and the supernatant was gathered, serving as plasma and stored at -80°C. The plasma was used for reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA).

2.3. Isolation of DNA and quantification of mtDNA

The mtDNA was isolated from the plasma with a DNeasy Blood and Tissue Kit (#69504; Qiagen). Initially, plasma (50 μ L) was mixed with equal volume of phosphate buffered saline. And the mixture was centrifuged at 16,000g, 4°C for 15 min. The supernatant (90 μ L) of which was reserved and proceeded to the following steps based on the protocol. Afterwards, 200 μ L elution buffer (EB) was loaded to dissolve DNA.

The plasmid carrying human mtDNA's complementary sequence and obtained from ORIGENE (SC101172; USA) was diluted as 10-fold serial system before being detected to generate a standard curve. Due to this particular plasmid, plasma mtDNA level was able to be quantified by a SYBR green dye-based RT-PCR assay reliant on the PRISM 7300 sequence detection system. The sequences of primers were designed from human NADH dehydrogenase I gene: CGAGCAGTAGCCCAACAAT (forward) and TGTGATAAGGGTGGAGAGGTT (reverse). After that, plasma mtDNA's concentration was converted through a special calculator and was displayed in copies per microliter plasma (<http://cels.uri.edu/gsc/cndna.html>; University of Rhode Island Genomics and Sequencing Center).¹²

Alongside the standard curve, all samples were measured at the same time and then were advanced to the formula.

$$c = Q \times V_{DNA} / V_{PCR} \times 1 / V_{ext}$$

In which c stands for the concentration of plasma mtDNA (copies/ μ L), Q for the quantity of DNA detected by RT-PCR, V_{DNA} and V_{PCR} for the volume of plasma DNA from the extraction and RT-PCR respectively, V_{ext} for the volume of plasma applied for the extraction. In this case, 200 μ L V_{DNA} , 1 μ L V_{PCR} and 50 μ L V_{ext} were employed.

2.4. Measurement of cytokines

Plasma TNF- α (DTA00C), IL-6 (D6050), and IL-8 (D8000C) levels were measured by Spectrophotometry (VARIOSKAN, Thermo, USA) and calculated using ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Samples were assayed in triplicate. Serial diluted standards were performed at the same time.

2.5. Statistical analysis

Data are presented as numbers, percentages, and means \pm standard deviations. Continuous variables were compared using the *t* test for normally distributed data. Correlations between mtDNA and inflammatory mediators were analyzed using Pearson correlation coefficient. Two-tailed *P* values < 0.05 were considered statistically significant. The data analysis was performed using a commercially available statistical software package (SPSS II for Windows, ver 16.0; SPSS, Chicago, IL, USA).

3. Results

3.1. Patient demographics

The demographics of the enrolled subjects are shown in Table 1. The mean age at surgery was 4.4 months, while the mean weight was 5.8 kg. Nine subjects underwent emergency surgery, while the others underwent elective surgery. The CPB time was 96.5 \pm 27.3 min and aortic cross-clamping time was 58.7 \pm 15.4 min. The mean interval from surgery to intensive care unit discharge was 3.4 \pm 1.2 days, while the post-operative mechanical ventilation time was 1.6 \pm 0.7 days.

3.2. Dynamic changes in plasma mtDNA and inflammatory mediators

As shown in Fig. 1, the plasma mtDNA level was elevated at the end of CPB (T2) (*P* < 0.05) and peaked at 6 h after CPB (T3) (*P* < 0.05). Similarly, Fig. 2 shows a significant increase in the plasma levels of TNF- α and IL-6 at T2 and peaks at T3 (*P* < 0.05). The IL-8 level peaked at T2 (*P* < 0.05) and then declined.

3.3. Correlation between peak plasma mtDNA and inflammatory cytokines

Significant positive correlations were found between peak plasma mtDNA level (at T3) and several inflammatory biomarkers, including IL-6 (at T3) (*r* = 0.62, *P* < 0.001), IL-8 (at T2) (*r* = 0.53, *P* < 0.001), and TNF- α (at T3) (*r* = 0.61, *P* < 0.001) (Fig. 3A–C).

Table 1
Baseline information

Characteristics	N = 48
Age, month	4.4 \pm 2.3
Sex	
Male	27
Female	21
Weight (kg)	5.8 \pm 2.2
Urgency of surgery	
Urgent	9
Elective	39
Information of surgery and ICU	
Cardiopulmonary Bypass Time (min)	96.5 \pm 27.3
Aortic Cross-clamping Time (min)	58.7 \pm 15.4
Postoperative mechanical ventilation(day)	1.6 \pm 0.7
Length of ICU stay(day)	3.4 \pm 1.2
ICU: intensive care unit	

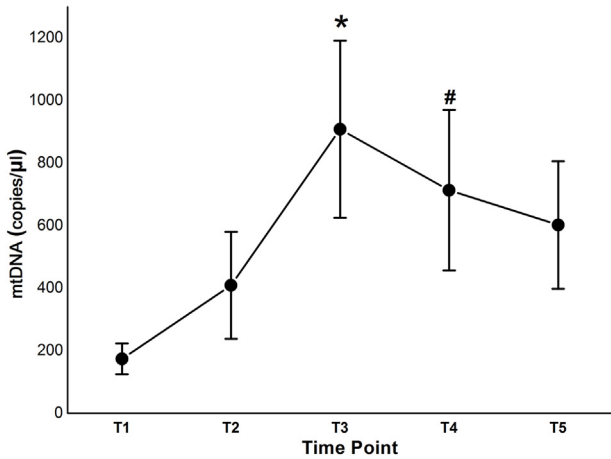


Fig. 1. Perioperative plasma mtDNA levels (pg/ml) in infants undergoing ventricular septal defect closure using CPB. Plasma mtDNA levels after the end of CPB were significantly higher than at T1. The plasma mtDNA level peaked at T3. The plasma mtDNA level at T4 was lower than that at T3 but still higher than that at T1. * $P < 0.05$ vs. T1. # $P < 0.05$ vs. T4.

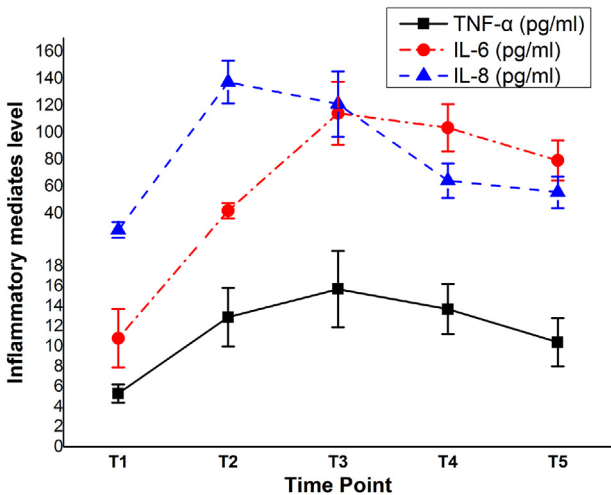


Fig. 2. Merged curves of perioperative inflammatory cytokine levels of infants undergoing ventricular septal defect closure by CPB. All inflammatory cytokine levels were significantly elevated after CPB ($P < 0.05$). TNF- α and IL-6 levels peaked at T3, while IL-8 levels peaked at T2 ($P < 0.05$).

4. Discussion

In this study of infants who underwent cardiac surgery to close ventricular septal defects, we demonstrated for the first time that mtDNA was involved in the systemic inflammatory response induced by CPB. Plasma mtDNA level was increased at the end of CPB and peaked at 6 h after CPB. We further revealed a close relationship between the mtDNA and inflammatory mediators (TNF- α , IL-6, and IL-8). Our novel findings suggest an important role of mtDNA in the development of the systemic inflammatory response induced by CPB.

CPB is reportedly remarkably associated with the systemic inflammatory response, which involves elevated levels of inflammatory mediators such as TNF- α , IL-6, and IL-8.⁵ TNF- α might play an important role in hypotension, coagulopathy, and renal dysfunction.^{13–15} TNF- α and IL-8 are also associated with capillary leakage and subsequent pulmonary edema, which may lead to lung injury.¹⁶ IL-6 is mainly secreted by fibroblasts, macrophages, lymphocytes, mesangial cells, and endothelial cells, and it plays an important role in regulating the inflammatory processes that occur during CPB.¹⁷ In the present study, we detected the plasma concentrations of TNF- α , IL-6, and IL-8 in infants on CPB undergoing ventricular septal defect closure and demonstrated increasing levels after CPB. We noted a systemic inflammatory response to CPB.

Qin et al. revealed mtDNA was related to inflammatory response in CPB of adults who underwent coronary artery bypass grafting (CABG).¹⁸ Here we determined for the first time that mtDNA was involved in the inflammatory response to CPB of infantile patients. It is well known that mtDNA is released after trauma surgery and that its release occurs in a cell necrosis-independent manner.¹⁹ In our study, after surgery using CPB, we found that plasma mtDNA levels increased after the end of CPB and peaked at 6 h post-CPB, then decreased gradually to a level that was higher than the baseline level. Cardiac I/R injuries reportedly induce the inflammatory response that is considered responsible for the high incidence of poor outcomes following CPB.²⁰ Our results suggest that elevation of plasma mtDNA might indicate I/R injury-induced inflammatory responses. After CPB, it is understood that post-I/R caused secondary damage to the heart.²¹ Our study shows that elevated plasma mtDNA level may indicate this secondary I/R injury-induced damage.

As a pro-inflammatory agent, mtDNA has been studied in many different fields and confirmed to cause inflammatory responses.^{22,23} Zhang Q et al. reported that plasma mtDNA, a kind of mitochondrial damage-associated molecular pattern, can induce severe inflammatory responses and result in a sepsis-like condition.¹¹ Our study found that the release of mtDNA after cardiac surgery with CPB was positively correlated with

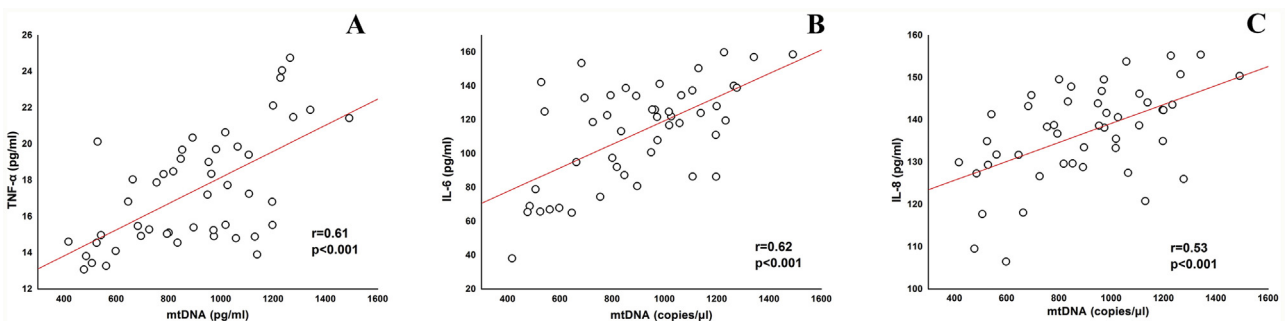


Fig. 3. Correlation between peak plasma mtDNA and peak inflammatory cytokine levels (the red lines indicate the positive correlation). (A) Scatter graph of a bivariate analysis demonstrating a positive correlation between peak plasma mtDNA and peak TNF- α levels ($r = 0.61$, $P < 0.001$). (B) Scatter graph of a bivariate analysis demonstrating a positive correlation between peak plasma mtDNA and peak IL-6 levels ($r = 0.62$, $P < 0.001$). (C) Scatter graph of a bivariate analysis demonstrating a positive correlation between peak plasma mtDNA and peak IL-8 levels ($r = 0.53$, $P < 0.001$).

inflammatory cytokine levels, suggesting that mtDNA may play an important role in inflammatory responses to CPB. Our data provides a new insight to further understand the mechanism of post-CPB inflammation, although further studies are needed to provide more details about mtDNA-related mechanisms and effects.

5. Study limitations

There are several limitations in the present study. First, the study population was small. However, we compared the mtDNA and inflammatory mediators among different time points in the same group to ensure the comparability of the data. Secondly, the present study did not demonstrate the source of mtDNA which need more research to find out. Although Qin et al.²⁴ had further investigated the activated platelets as one of the possible source of mtDNA after CPB, we need more studies to discuss the possibilities of endothelial dysfunction, immune activation and direct damage of heart tissue after CPB.

6. Conclusion

In this small sample size, prospective study, we demonstrated that the concentrations of mtDNA and inflammatory mediators in infants increase substantially after CPB. We also presented a novel finding that mtDNA may cause a systemic inflammatory response to CPB. Future therapeutic strategies for CPB-related inflammation and adverse events might benefit from the ability to decrease the amount of mtDNA released into the circulation.

References

- Guzzetta NA, Allen NN, Wilson EC, Foster GS, Ehrlich AC, Miller BE. Excessive postoperative bleeding and outcomes in neonates undergoing cardiopulmonary bypass. *Anesth Analg*. 2015;120(2):405–410.
- He S, Lin K, Ma R, Xu R, Xiao Y. Effect of the urinary trypsin inhibitor ulinastatin on cardiopulmonary bypass-related inflammatory response and clinical outcomes: a meta-analysis of randomized controlled trials. *Clin Ther*. 2015;37(3):643–653.
- Desai NK, Hamrick SE, Strickland MJ, Matthews E, McMaster L, Mahle WT. White matter injury and the inflammatory response following neonatal cardiac surgery. *Pediatr Cardiol*. 2015;36(5):942–949.
- Atkins BZ, Danielson DS, Fitzpatrick CM, Dixon P, Petersen RP, Carpenter AJ. Modified ultrafiltration attenuates pulmonary-derived inflammatory mediators in response to cardiopulmonary bypass. *Interact Cardiovasc Thorac Surg*. 2010;11(5):599–603.
- Mahle WT, Matthews E, Kanter KR, Kogon BE, Hamrick SE, Strickland MJ. Inflammatory response after neonatal cardiac surgery and its relationship to clinical outcomes. *Ann Thorac Surg*. 2014;97(3):950–956.
- el Habbal MH, Carter H, Smith LJ, Elliott MJ, Strobel S. Neutrophil activation in paediatric extracorporeal circuits: effect of circulation and temperature variation. *Cardiovasc Res*. 1995;29(1):102–107.
- Volk T, Schmutzler M, Engelhardt L, Docke WD, Volk HD, Konertz W, Kox WJ. Influence of aminosteroid and glucocorticoid treatment on inflammation and immune function during cardiopulmonary bypass. *Crit Care Med*. 2001;29(11):2137–2142.
- Kohira S, Oka N, Inoue N, Itatani K, Kitamura T, Horai T, Oshima H, Tojo K, Yoshitake S, Miyaji K. Effect of additional preoperative administration of the neutrophil elastase inhibitor sivelestat on perioperative inflammatory response after pediatric heart surgery with cardiopulmonary bypass. *Artif Organs*. 2014;38(12):1018–1023.
- Wallace DC. Mitochondrial DNA in aging and disease. *Sci Am*. 1997;277(2):40–47.
- Mittra I, Nair NK, Mishra PK. Nucleic acids in circulation: are they harmful to the host? *J Biosci*. 2012;37(2):301–312.
- Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464(7285):104–107.
- Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, Quintana C, Osorio JC, Wang Z, Zhao Y, Lawler LA, Christie JD, Meyer NJ, Mc Causland FR, Waikar SS, Waxman AB, Chung RT, Bueno R, Rosas IO, Fredenburgh LE, Baron RM, Christiansi DC, Hunninghake GM, Choi AM. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. *PLoS Med*. 2013;10(12):e1001577 [discussion e1001577].
- Bertani T, Abbate M, Zoja C, Corna D, Perico N, Ghezzi P, Remuzzi G. Tumor necrosis factor induces glomerular damage in the rabbit. *Am J Pathol*. 1989;134(2):419–430.
- Gomez-Chiarri M, Ortiz A, Gonzalez-Cuadrado S, Seron D, Emancipator SN, Hamilton TA, Barat A, Plaza JJ, Gonzalez E, Egidio J. Interferon-inducible protein-10 is highly expressed in rats with experimental nephrosis. *Am J Pathol*. 1996;148(1):301–311.
- Brennan DC, Yui MA, Wuthrich RP, Kelley VE. Tumor necrosis factor and IL-1 in New Zealand Black/White mice. Enhanced gene expression and acceleration of renal injury. *J Immunol*. 1989;143(11):3470–3475.
- Miakotina OL, Snyder JM. TNF-alpha inhibits SP-A gene expression in lung epithelial cells via p38 MAPK. *Am J Physiol Lung Cell Mol Physiol*. 2002;283(2):L418–L427.
- Greenberg JH, Whitlock R, Zhang WR, Thiessen-Philbrook HR, Zappitelli M, Devarajan P, Eikelboom J, Kavsak PA, Devereaux PJ, Shortt C, Garg AX, Parikh CR, Consortium T-A. Interleukin-6 and interleukin-10 as acute kidney injury biomarkers in pediatric cardiac surgery. *Pediatr Nephrol*. 2015;30(9):1519–1527.
- Qin C, Liu R, Gu J, Li Y, Qian H, Shi Y, Meng W. Variation of perioperative plasma mitochondrial DNA correlate with peak inflammatory cytokines caused by cardiac surgery with cardiopulmonary bypass. *J Cardiothorac Surg*. 2015;10:85.
- Itagaki K, Kaczmarek E, Lee YT, Tang IT, Isal B, Adibnia Y, Sandler N, Grimm MJ, Segal BH, Otterbein LE, Hauser CJ. Mitochondrial DNA released by trauma induces neutrophil extracellular traps. *PLoS One*. 2015;10(3):e0120549.
- Holmberg FE, Ottas KA, Andreassen C, Perko MJ, Moller CH, Engstrom T, Steinbruechel DA. Conditioning techniques and ischemic reperfusion injury in relation to on-pump cardiac surgery. *Scand Cardiovasc J*. 2014;48(4):241–248.
- Steinbrenner H, Bilgic E, Pinto A, Engels M, Wollschlager L, Dohrn L, Kellermann K, Boeken U, Akhyari P, Lichtenberg A. Selenium pretreatment for mitigation of ischemia/reperfusion injury in cardiovascular surgery: influence on acute organ damage and inflammatory response. *Inflammation*. 2016;39(4):1363–1376.
- Cao H, Ye H, Sun Z, Shen X, Song Z, Wu X, He W, Dai C, Yang J. Circulatory mitochondrial DNA is a pro-inflammatory agent in maintenance hemodialysis patients. *PLoS One*. 2014;9(12):e113179.
- Wilkins HM, Carl SM, Weber SG, Ramanujan SA, Festoff BW, Linseman DA, Swerdlow RH. Mitochondrial lysates induce inflammation and Alzheimer's disease-relevant changes in microglial and neuronal cells. *J Alzheimers Dis*. 2015;45(1):305–318.
- Qin C, Gu J, Hu J, Qian H, Fei X, Li Y, Liu R, Meng W. Platelets activation is associated with elevated plasma mitochondrial DNA during cardiopulmonary bypass. *J Cardiothorac Surg*. 2016;11(1):90.