Combined Effect of Telomere Length and Mitochondrial DNA Copy Number As a Potential Biomarker Indicating PE Risk: a Case-Control Study in a Chinese Population

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Abstract

Purpose To explore changes of Telomere length (TL) and mitochondrial copy number (mtDNA-CN) in preeclampsia (PE) and to evaluate the combined effect of maternal TL and mtDNA-CN on PE risk.

Methods A case-control study of 471 subjects (130 PE cases and 341 age frequency matched controls) was conducted in Nanjing Drum Tower Hospital, Jiangsu Province of China. Relative telomere length (RTL) and mtDNA-CN were measured using quantitative polymerase chain reaction (qPCR) and PE risk was calculated between groups by logistic regression analyses.

Results PE patients displayed longer RTL (0.48 versus 0.30) and higher mtDNA-CN (3.02 versus 2.00) in maternal blood as well as longer cord blood RTL (0.61 versus 0.35) but lower mtDNA-CN (1.69 versus 5.49) in cord blood (all $p < 0.001$). Exercise during pregnancy exerted an obvious effect of prolonging maternal telomere length. Multiparous, women with folic acid intake during early pregnancy and those delivered vaginally showed longer telomere length while those factors imposed no or opposite effect on RTL in PE cases. Furthermore, RTL and mtDNA-CN were positively correlated in controls (in maternal blood $r = 0.18$, $p < 0.01$; in cord blood $r = 0.19$, $p < 0.001$), but this correlation was disrupted in PE cases, no matter in maternal blood or in cord blood. Longer maternal RTL and higher mtDNA-CN were associated with higher risk of PE, and the ROC curve of RTL and mtDNA-CN in predicting PE risk presented an AUC of 0.755 (95%CI: 0.698-0.812).

Conclusions Interaction of TL and mtDNA-CN may play an important role in pathogenesis of PE and it could be a potential biomarker indicating PE risk.

Introduction

Preeclampsia (PE), a serious multisystem disorder, is defined as a new onset of hypertension with either proteinuria or end-organ dysfunction after 20 weeks of gestation [1]. With an incidence of 2-8% worldwide, PE is one of the leading causes of maternal and perinatal morbidity and mortality [1]. Although the pathophysiology of PE is not completely understood, early poor perfusion or placental ischemia-reperfusion injury leading to increased oxidative stress has been widely accepted as one of the main pathological processes responsible for the development of PE [2–3]. There are some markers of oxidative stress raised in PE circulation [4], but it is not well established about the specific changes of oxidative stress biomarkers in the context of PE and insights into the relationship between oxidative stress markers and PE will help to illustrate the mechanisms of oxidative stress in the context of PE and to discuss perspectives on disease prediction.

Telomere length (TL) and mtDNA copy number (mtDNA-CN) are emerging biomarkers of oxidative stress and have been related to various age-related diseases. They are highly susceptible to oxidative stress [5] and inflammation so that have been suggested as sensitive indexes of cellular oxidative stress, mitochondrial dysfunction, aging process, and age-related diseases [6]. Recent research has indicated that telomeres and mitochondria are functionally linked and telomere dysfunction could induce p53 represses PGCs and result in metabolic and mitochondrial compromise, suggesting disorder of telomere-mitochondria axis may be an important and early event in biological aging diseases [7–8]. Hyunsu et al found that loss of the association between telomere length and mitochondrial DNA copy number may induce the initiation of Colorectal Carcinogenesis and co-regulation of telomere and mitochondria may play an important role in the genesis and development of Oxidative stress-related diseases [9]. However, evidence referring to TL and mtDNA-CN in preeclampsia was scarce and the results were mixed [10–11].

Therefore, we hypothesized that alterations in leukocyte TL and mtDNA-CN may reflect the cumulative exposure to oxidative stress and underlie the pathogenesis of PE. However, no previous study has been conducted on the association between TL and mtDNA-CN in PE cases. Here, we designed a case-control study with 130 PE cases and 341 controls in Chinese population to investigate TL and mtDNA-CN alterations in maternal peripheral blood and cord blood in women with PE, and further to explore their combined effect of on PE risk. The results of present study may aid improvement in the current understanding of PE, through identifying the joint involvement of TL and mtDNA-CN in PE pathogenesis.

Materials And Methods

Study recruitment

PE cases and controls were recruited from Nanjing Drum Tower Hospital in Nanjing, Jiangsu province of eastern China between January 2019 and June 2020. According to the report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy, PE is diagnosed by specialist doctors at admission [12]. During the same period, women who were healthy without any complications and age frequency matched were recruited as controls. Inclusion criteria of the study were single viable pregnancy, any maternal age, maternal BMI, and parity status. Exclusion criteria were multiple pregnancies, pregnancy with fetal anomalies, pregnancy with preexisting chronic disease such as chronic hypertension, diabetes mellitus et al and other pregnancy complications such as gestational diabetes, prelabor rupture of membranes, chorioamnionitis, placenta abruptions and so on. Finally, 130 cases and 341 controls were included. All subjects were informed about the study and after signing the informed consent; they were interviewed by well-trained interviewers with structured questionnaires. Data available in the questionnaire include demographic data and lifestyle related factors during pregnancy such as education, folic acid intake, occurrence of threatened abortion, physical activity, secondhand smoking, BMI at labor admission, medical and reproductive characteristics. Delivery related information such as gestational age, delivery mode, birth weight, and occurrence of postpartum hemorrhage (PPH) were obtained from obstetric records. Gestational age was calculated based on last menstrual period or ultrasound-based estimated date of conception. Both physical activity and secondhand smoke were divided into four groups by levels of activities.

Maternal peripheral venous blood samples were collected from 471 participants right before or during delivery. Paired umbilical cord blood samples were collected immediately after birth from the cord vein of newborns. Blood samples were shipped by cold chain equipment to the laboratory and stored until analysis. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the ethics committees of Nanjing Drum Tower Hospital (No.2018-017).

Measurement of relative telomere length and mitochondrial DNA copy number

Genomic DNA was extracted from leukocytes of maternal peripheral blood and cord blood. Telomere length and mtDNA-CN were analyzed on modified quantitative polymerase chain reaction (qPCR) by QuantStudio 7TM FlexReal-Time PCR System (Applied Biosystems). The ratio of telomere repeat copy number (T) to single copy gene 36B4 number (S) was computed to reflect the relative telomere length (RTL). Primers sequences for telomere PCR were TEL1, 5'-

GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3' and TEL2, 5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3' and the single-copy gene (36B4) primers sequences were 36B4u, 5'-CAGCAAGTGGGAAGGTGTAATCC-3', and 36B4d, 5'-

CCCATTCTATCATCAACGGGTACAA-3'. We also determined mtDNA-CN as the ratio of mitochondrial encoded NADH dehydrogenase-1 (ND-1) to nuclear gene (hemoglobin subunit β , HGB) by simultaneous amplifications of ND1 and HGB genes.

Primers sequences were as follows: ND1 forward 5'-CCCTAAACCCGCCACATCT-3'; ND1 reverse 5'-

GAGCGATGGTGAGAGCTAAGGT-3'; HGB forward 5'-GAAGAGCCAAGGACAGGTAC-3', and HGB reverse 5'-

CAACTTCATCCACGTTTACC-3'. Reference DNA (pooled from 5 healthy controls) was used to generate a standard curve for quantification. After exclusions of outliers, average cycle threshold (Ct) values of the remain samples were calculated. Each reaction system contains 10 μ l SYBR $\text{\textcircled{R}}$ Green PCR Master Mix (Applied Biosystems) with a final DNA concentration of 5 ng/ μ l. All samples were assayed in duplicate and three quality controls were employed in each plate to analyze variability. qPCRs were executed by investigators blinded to clinical data and disease status. RTL and mtDNA-CN were calculated based on Cawthon's formula [13–14]:

$$2^{-(\Delta Ct1 - \Delta Ct2)} = 2^{-\Delta \Delta Ct}$$

Statistical analysis

All statistical analyses were performed with Stata version 15.1 (Stata Corp, College Station, TX). Sample characteristics were described as means (SD), median (IQR) or percentages. Pearson χ^2 test was used to test differences between cases and controls for categorical variables (maternal age group, education, folic acid intake, occurrence of threatened abortion, physical activity, secondhand smoke exposure status, BMI at delivery and mode of delivery). T-test and Wilcoxon rank test were employed to compare the differences of normal distributed and nonparametric continuous variables in cases and controls. Wilcoxon signed rank test was utilized for the comparisons of RTL and mtDNA-CN between matched maternal blood and cord blood. Kruskal Wallis rank test was used to compare nonparametric variables among groups. Correlations of RTL and mtDNA-CN in PE cases and controls were analyzed by Spearman's rank correlation. To estimate the relative association between preeclampsia and levels of maternal RTL and mtDNA-CN, we categorized maternal RTL and mtDNA-CN into two groups according to their median distribution. The odds ratios (ORs) and 95% confidence intervals (95% CIs) of RTL and mtDNA-CN associated with PE risk were calculated by logistic regression analyses. Then a backward stepwise logistic regression was carried out to explore the independent factors on PE risk. Receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC) were calculated to estimate the feasible use of maternal RTL and mtDNA-CN as possible markers in determining PE risk. A two-sided $P < 0.05$ was considered statistically significant, and p values with significance are marked in bold in tables.

Results

Baseline characteristics of study participants in PE cases and controls

Women with PE tended to be less educated, exerted with more gravidities, lower folic acid intake rate, higher threatened abortion rate in early pregnancy, more secondhand smoke exposure, smaller gestational age, higher BMI at delivery, higher cesarean section rate, lower birth weight, higher PPH incidence yet with an increase in physical activity compared to the controls ($p < 0.05$). No significant differences were found for parity between cases and controls. Maternal RTL was significantly longer in PE patients as compared with controls (median: 0.48 versus 0.30; $p < 0.001$), and this trend was also observed from cord blood (median: 0.61 versus 0.35; $P < 0.001$). Maternal mtDNA-CN of PE cases was significantly higher than that of controls (median: 3.02 versus 2.00; $p < 0.001$); however, in cord blood, lower mtDNA-CN was found in PE cases (median: 1.69 versus 5.49; $p < 0.001$) (Table 1. Fig. 1).

Table 1
Characteristics of study participants in PE cases and controls

	PE cases	Controls	p value
N	130	341	
Maternal age (years)			
<25	14(10.77)	27(7.92)	0.052
25~29	56(43.08)	179(52.49)	
30~34	38(29.23)	104(30.50)	
>=35	22(16.92)	31(9.09)	
Education			
Low	46(35.38)	59(17.30)	<0.001
Middle	53(40.77)	140(41.06)	
High	31(23.85)	142(41.64)	
Gravidity	1.98+0.09	1.68+0.05	0.002
Parity	1.22+0.04	1.14+0.02	0.05
Folic acid intake			
None	33(25.38)	29(8.50)	<0.001
Sometimes	29(22.31)	87(25.51)	
Nearly everyday	68(52.31)	225(65.98)	
Threatened abortion			
No	102(78.46)	313(91.79)	<0.001
Yes	28(21.54)	28(8.21)	
Exercise			
None	42(32.31)	184(53.96)	<0.001
1-2 times per week	34(26.15)	98(28.74)	
3-4 times per week	32(24.62)	38(11.14)	
5-7 times per week	22(16.92)	21(6.16)	
Second hand smoking			
None	64(49.23)	235(68.91)	<0.001
Less than 0.5 hour a day	27(20.77)	79(23.17)	
0.5-1 hour a day	27(20.77)	15(4.40)	
More than 1 hour a day	12(9.23)	12(3.52)	
Gestational age	37.20+0.28	39.19+0.13	<0.001
BMI at delivery			
Quantile 1	30(23.08)	89(26.25)	<0.001
Quantile 2	20(15.38)	96(28.32)	

	PE cases	Controls	p value
Quantile 3	30(23.08)	87(25.66)	
Quantile 4	50(38.46)	67(19.76)	
Delivery mode			
Vaginal delivery	45(34.62)	289(84.75)	<0.001
Caesarean section	85(65.38)	52(15.25)	
Birth weight	2827.08+-69.85	3303.19+-30.26	<0.001
Postpartum Hemorrhage			
No	78(60.00)	279(81.82)	<0.001
Yes	52(40.00)	62(18.18)	
Materl blood RTL	0.48(0.27-0.77)	0.30(0.24-0.36)	<0.001
Cord blood RTL	0.61(0.35-1.28)	0.35(0.29-0.42)	<0.001
Maternal blood mtDNA-CN	3.02(1.83-4.89)	2.00(1.15-3.31)	<0.001
Cord blood mtDNA-CN	1.69(0.91-3.60)	5.49(2.89-10.15)	<0.001

Comparisons of maternal RTL and mtDNA-CN in subgroups of PE cases and controls

As shown in Table 2, median RTL and mtDNA-CN in maternal blood were not influenced by maternal age, education, previous pregnancy, threatened abortion, preterm delivery, BMI at delivery, birth weight, and occurrence of PPH. Participants with more exercise during pregnancy were tended to have longer maternal RTL (median: 0.76 versus 0.35 in cases and 0.32 versus 0.30 in controls, both $p < 0.05$). In controls, multiparous, women with folic acid intake during early pregnancy and those delivered vaginally showed longer telomere length while those factors imposed no or opposite effect on RTL in PE cases. Smoke exposure did not impact RTL in cases and controls but displayed an effect of increasing mtDNA-CN in controls.

Table 2
Comparisons of maternal RTL and mtDNA-CN in subgroups of PE cases and controls

Comparisons of maternal RTL and mtDNA-CN in subgroups of PE cases and controls										
PE cases						Contrlos				
Characteristics	n	RTL	p value	mtDNA-CN	p value	n	RTL	p value	mtDNA-CN	p value
Overall	130					341				
Maternal age (year)			0.1096		0.3575			0.8179		0.475
<25	14	0.56(0.42-0.77)		1.70(1.29-3.66)		27	0.30(0.25-0.36)		2.35(1.58-3.93)	
25~29	56	0.44(0.26-0.73)		3.17(1.52-5.02)		179	0.29(0.23-0.37)		1.86(1.02-3.09)	
30~34	38	0.41(0.26-0.82)		3.30(2.06-4.63)		104	0.31(0.25-0.35)		2.05(1.19-3.21)	
>=35	22	0.69(0.46-0.83)		2.94(2.09-5.49)		31	0.32(0.23-0.40)		2.23(1.03-4.42)	
Education			0.1522		0.0972			0.928		0.9138
Low	46	0.62(0.29-0.85)		2.26(1.38-4.77)		59	0.30(0.23-0.40)		2.17(1.05-3.52)	
Middle	53	0.45(0.25-0.74)		3.42(2.12-5.32)		140	0.30(0.24-0.37)		2.01(1.16-3.54)	
High	31	0.41(0.28-0.62)		3.03(1.92-4.16)		142	0.30(0.25-0.36)		1.98(1.15-3.25)	
Previous pregnancy			0.1946		0.3067			0.0572		0.2386
No	51	0.43(0.27-0.70)		2.96(1.66-4.30)		189	0.29(0.24-0.36)		2.04(1.27-3.31)	
Yes	79	0.55(0.28-0.82)		3.38(1.84-5.28)		152	0.31(0.24-0.38)		1.96(1.03-3.37)	
Parity			0.1385		0.5436			0.0029		0.555
Primiparous	102	0.45(0.27-0.73)		3.02(1.72-4.73)		291	0.29(0.24-0.36)		2.00(1.16-3.45)	
Multiparous	28	0.59(0.31-0.87)		3.21(1.88-5.54)		50	0.34(0.30-0.39)		2.01(1.04-3.05)	
Folicacid intake			0.0281		0.6406			0.0369		0.5704
No	33	0.67(0.39-0.88)		3.51(1.72-4.51)		29	0.26(0.22-0.32)		2.38(1.10-4.62)	
Yes	97	0.45(0.27-0.73)		2.94(1.83-5.02)		312	0.30(0.25-0.37)		1.99(1.15-3.25)	
Threatened abortion			0.9862		0.1643			0.3249		0.6863
No	102	0.48(0.27-0.82)		2.90(1.84-4.51)		313	0.30(0.24-0.36)		1.99(1.15-3.52)	
Yes	28	0.47(0.29-0.73)		4.01(1.64-6.11)		28	0.30(0.26-0.37)		2.12(1.12-2.96)	

PE cases				Contrlos				
Exercise			<0.001		0.5665		0.0309	<0.001
None or seldom	76	0.35(0.24-0.49)		3.01(1.92-5.04)		282	0.30(0.24-0.36)	2.18(1.31-3.57)
Often	54	0.76(0.51-0.97)		3.04(1.57-4.67)		59	0.32(0.23-0.48)	1.03(0.95-2.12)
Second hand smoking			0.0761		0.8427		0.8967	0.0393
None	64	0.45(0.28-0.76)		2.84(1.61-4.72)		235	0.30(0.24-0.36)	1.96(1.05-3.37)
Less than 0.5hour a day	27	0.37(0.22-0.70)		3.17(1.85-6.22)		79	0.29(0.24-0.36)	1.84(1.01-2.86)
0.5-1 hour a day	27	0.60(0.34-0.86)		2.57(1.96-4.87)		15	0.33(0.22-0.43)	2.67(2.16-5.57)
More than 1 hour a day	12	0.71(0.33-0.81)		3.64(1.29-5.77)		12	0.28(0.22-0.38)	2.38(1.84-3.41)
Preterm delivery			0.9717		0.3489		0.5142	0.3924
No	86	0.47(0.28-0.73)		2.75(1.76-4.74)		313	0.30(0.24-0.37)	1.98(1.12-3.22)
Yes	44	0.49(0.27-0.81)		3.67(1.92-5.04)		28	0.29(0.24-0.36)	2.67(1.29-3.69)
BMI at delivery			0.2521		0.4288		0.2482	0.8637
Quantile 1	30	0.70(0.26-0.91)		3.78(1.86-4.88)		89	0.29(0.23-0.34)	1.99(1.28-3.00)
Quantile 2	20	0.52(0.28-0.77)		2.01(1.38-3.20)		96	0.30(0.24-0.37)	2.20(1.18-3.37)
Quantile 3	30	0.46(0.37-0.79)		3.45(2.21-4.87)		87	0.30(0.24-0.37)	1.77(1.05-3.32)
Quantile 4	50	0.43(0.26-0.66)		3.01(1.92-5.04)		67	0.31(0.25-0.37)	1.87(1.03-4.21)
Delivery mode			0.0105		0.2416		0.0308	0.1702
Vaginal delivery	45	0.40(0.26-0.62)		3.02(1.66-4.16)		289	0.30(0.24-0.37)	1.96(1.05-3.37)
Caesarean section	85	0.57(0.29-0.89)		3.01(1.88-5.31)		52	0.28(0.23-0.33)	2.25(1.42-3.25)
Low birth weight			0.311		0.7459		0.5765	0.1952
No	93	0.45(0.27-0.73)		2.95(1.84-5.00)		318	0.30(0.24-0.37)	1.98(1.10-3.30)
Yes	37	0.65(0.29-0.82)		3.51(1.68-4.89)		23	0.29(0.23-0.36)	2.69(1.38-3.52)
Postpartum hemorrhage			0.3484		0.459		0.4309	0.4442
No	78	0.45(0.27-0.73)		3.17(1.89-4.88)		279	0.30(0.24-0.37)	2.03(1.15-3.58)

PE cases				Contrlos		
Yes	52	0.55(0.28-0.81)	2.62(1.51-5.00)	62	0.29(0.24-0.35)	1.93(1.07-2.86)

Table 3

Odds ratios (OR) and 95% Confidence Interval (CI) for preeclampsia according to categories of maternal RTL and mtDNA-CN

Variables	PE cases	Controls	OR(95%CI) ^a	<i>p</i> ^a
	<i>N</i> (%)	<i>N</i> (%)		
Maternal RTL				0.006
Low(≤ 0.314)	40(31.25)	195(57.18)	1	
High(> 0.314)	88(68.75)	146(42.82)	2.02(1.22-3.32)	
Maternal mtDNA-CN				0.005
Low(≤ 2.20)	46(36.51)	186(55.03)	1	
High(> 2.20)	80(63.49)	152(44.97)	2.14(1.26-3.63)	
^a Logistic regression analysis, adjusted for age, BMI, gravidity, parity, folic acid intake, threatened abortion, second hand smoke, physical activity and education.				

Association between RTL and mtDNA-CN in maternal blood and cord blood

As shown in Fig. 2, a positive correlation of maternal RTL and mtDNA-CN was determined in normal pregnancy($r=0.18$, $p<0.01$, Fig. 2A), but this correlation was disappeared in PE patients ($p=0.52$, Fig. 2B). We also found positive correlation between RTL and mtDNA-CN in cord blood within controls($r=0.19$, $p<0.001$, Fig. 2C) while a negative correlation was observed in PE cases($r=-0.23$, $p<0.01$, Fig. 2D).

PE risk according to different categories of maternal RTL and mtDNA-CN

Logistic regression was used to estimate adjusted OR (aOR) and 95% CIs for PE risk with maternal RTL and mtDNA-CN. We separately categorized maternal RTL and mtDNA-CN into two groups according to their median distribution. When compared to those in the lower(shorter) group, significant elevated risk of PE was observed in both higher(longer) groups (RTL: aOR:2.02, 95%CI:1.22-3.32, $p=0.006$; mtDNA-CN: aOR: 2.14, 95%CI:1.26-3.63, $p=0.005$), after adjustment for maternal age, education, gravidity, parity, folic acid intake, threatened abortion, secondhand smoking, physical exercises and BMI at delivery (Tabel 3).

Effects of maternal RTL and mtDNA-CN for PE prediction

We further applied logistic regression models with backward stepwise procedures to investigate the independent factors associated with PE risk (Fig. 3). Model 1 incorporated all the maternal characteristics associated with PE from Table 1 while model 2 included only maternal RTL and mtDNA-CN. ROC curves and AUCs of the two models were calculated, respectively. Results revealed maternal RTL and mtDNA-CN were strong predictors of PE risk. Maternal RTL, mtDNA-CN, threatened abortion and second-hand smoking during pregnancy jointly showed significant predict value on PE risk with an AUC of 0.781 (95%CI: 0.726-0.836) in model 1 while model 2 with RTL and mtDNA-CN only presented an AUC of 0.755(95%CI: 0.698-0.812). There was no significant difference between these two models ($p>0.05$).

Discussion

Given the dearth of findings on the role of RTL and mtDNA-CN in the pathophysiology of PE, this study aimed to compare RTL and mtDNA-CN in PE patients and normal pregnant controls, in the meanwhile, to evaluate predictive value of maternal RTL and mtDNA-CN on PE risk. Our study demonstrated that PE patients displayed longer RTL and higher mtDNA-CN in maternal blood as well as longer cord blood RTL, but lower cord blood mtDNA-CN compared to normal pregnant women. Multiparous, exercise during pregnancy and first trimester folic acid intake exerted lengthening effect on maternal telomere length. Furthermore, RTL and mtDNA-CN were positively correlated in healthy pregnant women and newborns while this correlation was disrupted in PE cases. We also found longer maternal RTL and higher mtDNA-CN were associated with higher risk of PE, followed by presenting the evidence that combination of maternal RTL and mtDNA-CN was effective in prediction of PE risk. Up to our knowledge, this was the first study to examine the combined prediction effect of maternal RTL and mtDNA-CN on PE risk.

Previous studies reported shorter telomeres in placental samples from pregnancies complicated with PE and suggested abnormal telomere homeostasis was closely related to the pathogenesis of PE and raised TL could be a potential biomarker of PE [15]. Evidence has supported consistency of telomere length from different tissue, thus easily accessible leukocyte was widely employed as a substitute of tissue in telomere length measurement [16–19]. However, research on peripheral leukocyte TL and PE was scarce. One previous study was conducted with 50 cases of PE and 50 controls in Washington State. Harville et al analyzed telomere length in peripheral blood and found women in the highest tertile showed a trend of higher PE risk (OR 1.08) compared to those in the lowest tertile when adjusted with age although the difference was not statistically significant [10], which may be explained by the limited sample size. As TL shortening has been associated with various aging related diseases, it is somewhat surprising that we found longer leukocyte TL was associated with PE in the current study. More physical activity among our PE patients may partially explain this inconsistency; most of our PE patients were more likely to follow doctors' advice during pregnancy and kept good lifestyles although they had been in initial abnormal state. Evidence supported that telomere length of leukocyte cells were positively associated with healthy living and physical activity may confer protection against telomere length shortening [20–22]. Thus, the modified life style may lengthen TL enough to compensate TL attrition when most PE cases in our study were mild and well controlled with imperceptible shortening. Furthermore, although in cells of most human tissue telomeres shorten throughout human life, it is heavily confounded by, among other factors, the variable levels of telomerase activity—and hence variable capacities for telomere length replenishment—in stem cells. These can constantly renew somatic tissue cells. Telomerase is enriched in germline lineage cells and embryonic stem cells [23–26]; previous data have reported that telomerase reverse transcriptase (TERT) and telomerase activity were significantly higher in preeclamptic placenta when compared with control group according to different gestational age [27–28], which may cause a lengthening effect of TL in PE cases as well. Additionally, despite consistency of telomere length among tissue, leukocyte TL may not completely reflect TL changes in placenta in PE cases. More studies are warranted to elucidate the correlations between leukocyte TL and placenta TL throughout the progression of PE.

Mitochondrial dysfunction contributing to the pathogenesis of preeclampsia has been proposed by Torbergson et al [29] and Widschwendter et al [30], the latter indicating that defects in trophoblastic mitochondria may be the initial step in the pathophysiological process of PE. In this study, we found higher mtDNA-CN were associated with higher PE risk, which corroborates previous findings [31]. Previous data also reported that the odds of preeclampsia were positively associated with maternal blood mtDNA-CN [32]. These results further support the idea that elevated peripheral blood mtDNA-CN may serve as a risk marker for PE. It has been demonstrated that mtDNA is highly susceptible to oxidative stress and mitochondrial damage, as reflected by changes in mtDNA-CN may alter mitochondrial gene expression and cause oxidative phosphorylation deficiency and surge of ATP by glycolysis [33]. In addition, oxidative stress, an important pathogenesis pathway involved in preeclampsia [34], may alter mitochondrial function and increase mtDNA-CN through several mechanisms, it is possible that elevated systematic reactive oxygen species (ROS) may impair or disrupt cellular components such as mitochondrial lipid membranes [35]. ROS may also influence mitochondrial function by impairing DNA and damaging electron chain transport; and a compensatory response to this cellular stress may lead to an increase in mtDNA-CN [33, 36]. This result is highlighted by experimental animal studies demonstrating increased mitochondrial damage and mtDNA-CN with increasing exposure to pro-oxidants [35]. These data altogether suggest that that association of increased mtDNA-CN with preeclampsia is biologically plausible. However, several other studies reported inconsistent findings. The heterogeneity in results between studies may be related to population diversity, lifestyle modifications, exposure levels, time windows and ability of mitochondrial DNA compensation [37–38].

The initial or newborn setting of TL represents a critically important characteristic of an individual's telomere biology system [39]. In our study, we found that RTL of cord blood were longer than that of maternal blood both in controls and PE cases. But we did not observe a reverse association between age and RTL in pregnant women. Our narrow age range may have accounted for this lack of association; the rate of changes in telomere length varies with age and is greatest in childhood and elderly while relatively stable in adults [40–41]. In addition, only around 10 percent of women were at or older than 35 and less than 10 percent were younger than 25 in our study, so the small sample in those subgroups may yield some deviation. The above reasons may dilute the adverse effects of aging on telomere length in the current study. Recent studies emphasized telomere length had association with diet and lifestyle determinants [42–44]. In our study, we also found both folic acid intake and regular exercise exerted prolonging effect on maternal TL in normal pregnancy, which further support the idea that TL could be adjusted by modifying lifestyles. This result underlined that TL was sensitive enough to reflect alterations of oxidative stress in pregnant women and could be a reliable biomarker of oxidative stress during pregnancy.

Although mtDNA-CN has been directly related to obesity in child population [45], we did not observe this effect in our study. Higher mtDNA-CN was shown associated with less exercise and more secondhand smoke in normal pregnancy in this study. The excessive oxidative stress generated by less exercise and more secondhand smoke may lead to increased mtDNA-CN synthesis as a compensatory mechanism to ensure cell survival. In addition to population diversity, variations in levels and duration of exposure to oxidative stress [37, 46] caused by genetic factors as well as environmental factors have a dual influence on mtDNA-CN. Mild oxidative stress may first respond to increase energy demands by increasing mtDNA-CN [36, 47], while continued high exposure oxidative stress may induce mtDNA damage and result in decreased or limited replication of mtDNA due to increased abundance of defective mitochondria [36, 38]. This duality in response to mild vs. excessive oxidative stress might also explain previous mixed findings on mtDNA content. It is worthy to note that umbilical cord blood mtDNA-CN in PE cases was much lower than that of controls, which was in contrast to our result of maternal blood; in all probability pregnancy is a period when newborns are extremely susceptible to oxidative stress as this time mitosis is highly active, with a result that mitochondria in cord blood of PE patients exceeded the extent of compensation and showed a decrease in copy number. This finding is in line with a recent study which observed air pollution during pregnancy was associated with decreased mtDNA content in cord blood and suggested heightened sensitivity to oxidative stress during the specific prenatal window of life stage [48].

In our control subjects, there were positive correlations between mtDNA-CN and telomere length in maternal blood and cord blood suggesting co-regulation of telomeres and mitochondrial function in mothers and newborns in normal pregnancy. Telomere length and mtDNA-CN have largely been examined as independent contributors to oxidative stress related diseases, yet there is growing evidence that these two markers are functionally linked or at least combining them together may better predict disease development. Several recent studies concerning school age children and healthy adults as well as elderly women reported a positive association between mtDNA-CN and telomere length [49-52]. Studies involving animal model and cell culture experiments have also shown that telomere dysfunction is associated with abnormal mitochondrial biogenesis and function [53-54]. However, just limited evidence with small sample size [55] has documented the dependence between these two biomarkers in pregnant women. Therefore, we found out strong correlations between RTL and mtDNA-CN when they have been measured both in maternal and cord blood during normal pregnancy. Mechanism of this association remains to be determined; mitochondria effects of p53 activation from telomere dysfunction [7] and TERT effects on mtDNA repair may be involved [56]; Telomere shortening can reciprocally lead to cellular mitochondrial endangerment and diminished mitochondrial biogenesis via diminution of PGC-1 α , the master regulator of mitochondrial biogenesis [7]. In addition, TERT, a catalytic subunit of telomerase with canonical role of telomere maintenance, contains both nuclear localization signal and mitochondrial targeting sequence, and might be transported from nuclei to mitochondria under increased oxidative stress conditions to regulate mitochondrial function and protect mtDNA from oxidative damage [57]. However, this association was not observed in PE cases implying that the pathways shared by regulating telomere length and mitochondrial biogenesis might be disturbed by the pathophysiology of PE. The present findings provide evidence that telomeres and mitochondria are co-regulated in normal pregnant women and their newborns, and this co-regulation was interrupted when PE occurs, with increased RTL and mtDNA-CN been associated with increased PE risk. In this sense, the pathophysiology of PE may play a role in the mechanisms regulating the association between telomere length and mtDNA-CN and the complex interplay between TL and mtDNA-CN could be a potential effective predicting factor of PE risk. Considering this, we firstly investigated the cumulative effect of TL and mtDNA-CN on PE risk. As a

result, we found that combination of maternal leukocyte mtDNA-CN and RTL can effectively predict the risk of PE, contributing to recent investigations concerning improvement in PE prediction models. Further studies are still needed to verify our results and more precise knowledge of the regulatory pathways governing interaction of RTL and mtDNA-CN to PE process is also necessary to delineate both in its onset and pathogenesis.

Our study has some strength. We are the first to investigate the combined effect of maternal RTL and mtDNA-CN on PE prediction and firstly revealed the disruption of positive dependence between these two biomarkers participate in the process of PE even without knowing the causative or just a compliance. Second, many lifestyle factors such as smoking status, folic acid intake, exercise during pregnancy as well as BMI before delivery, which may influence RTL and mtDNA-CN, were applied and adjusted for in the present study to examine the independent effect of RTL and mtDNA-CN on PE risk. Moreover, unlike other studies regarding BMI and pregnancy, we chose to use BMI before delivery rather than pre-pregnancy because this variable would have more of impact on RTL and mtDNA-CN measured in our study as it takes into account weight gain during pregnancy.

Several limitations also need to be acknowledged. First, we only measured RTL and mtDNA-CN before delivery and the alterations of RTL and mtDNA-CN in leukocytes during different stages of pregnancy and disease progression remain unclear. Second, our study is restricted to Han Chinese; the generalizability to other ethnic cohort needs further evaluation. Third, due to the sample size, our study treated all PE cases as a whole; types of PE need to be investigated separately as early onset preeclampsia processes different pathogenesis as that of late onset. Fourth, although we have incorporated a series of lifestyle related factors influencing RTL and mtDNA-CN into our study, many other factors such as stress, environmental pollutants and detailed diet habits were not involved as they are difficult to measure objectively. Finally, we only carried out the association analyses among RTL, mtDNA-CN, and risk of PE. The underlying mechanisms that account for pathways of leukocyte mtDNA content and RTL effect PE pathophysiology need further investigation.

Conclusions

In conclusion, loss of positive correlation between RTL and mtDNA-CN in our study may induce the initiation or progression of PE pathogenesis; additionally, maternal RTL and mtDNA-CN before delivery were positively associated with the risk of PE, suggesting that increased levels of maternal RTL and mtDNA-CN were risk factors of PE and their combined effect had a predictive efficacy for PE risk. This study demonstrates the contribution of interplay between RTL and mtDNA-CN to pathogenesis of PE and opens a new perspective for PE prediction.

Abbreviations

TL: Telomere Length; mtDNA-CN: Mitochondrial Copy Number; PE: Preeclampsia; RTL: Relative Telomere Length; qPCR: quantitative Polymerase Chain Reaction; PPH : Postpartum Hemorrhage; ORs: Odds Ratios; ROC : Receiver Operating Characteristic; AUC: Area Under the ROC Curve; TERT: Telomerase Reverse Transcriptase.

Declarations

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We acknowledge and appreciate the cooperation of study participants.

Authors' contributions

RYZ implemented data collection, lab work and manuscript writing. JBD participated in project development, lab work, statistical analysis and manuscript editing. ZDX participated in data collection, lab work and manuscript editing, YJ and LJ participated in data collection and analyzed the results. QW designed the study, provided guidance in statistical analysis and reviewed the manuscript for intellectual content. RYZ, JBD and ZDX contributed equally. Correspondence and requests for materials should be addressed to QW (email: shuishangfanyi@sina.com). All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are not publicly available to protect the participants' anonymity. But can be freely available from the corresponding author on reasonable request.

Conflict of interests

All authors declare that they have no conflict or competing interests.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Nanjing Drum Tower Hospital Ethics Committee approved the study (No.2018-017). All participants provide verbal and written informed consent. The participants have the right to withdraw from the study at any time.

Consent for publication

Not applicable.

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Figures

Figure 1

Relative telomere length (RTL) and mitochondrial DNA copy number (mtDNA-CN) in maternal blood and cord blood. (A) RTL from controls and PE cases in maternal blood and cord blood. (B) mtDNA-CN from controls and PE cases in maternal blood and cord blood.

Figure 2

Association of RTL and mtDNA-CN in maternal blood and cord blood from controls and PE cases. (A) RTL and mtDNA-CN in maternal blood from normal pregnancy. (B) RTL and mtDNA-CN in maternal blood from PE patients. (C) RTL and mtDNA-CN in cord blood from normal pregnancy. (D) RTL and mtDNA-CN in cord blood from PE patients.

Figure 3

ROC curves for PE risk prediction. Model 1 incorporated all related maternal characteristics from Table 1; model 2 included only maternal RTL and mtDNA-CN.