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Technical note

Placental mitochondrial DNA copy number is associated with reduced birth weight in women with placental malaria

Sukma Oktavianthi[a], Muhammad Fauzi[b], Leily Trianty[a], Hidayat Trimarsanto[a], Anom Bowolaksono[b], Rintis Noviyanti[a], Safarina G. Malik[a,*]

[a] Eijkman Institute for Molecular Biology, Jl. Diponegoro No. 69, Jakarta, 10430, Indonesia
[b] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, 16424, Indonesia

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ABSTRACT

Placental malaria (PM) causes placental insufficiency, leading to reduced birth weight (BW). Placental mtDNA copy number (mtDNA-CN) and relative telomere length (RTL) have been described as potential biomarkers for placental insufficiency and intrauterine growth restriction (IUGR). We investigated their associations with BW in women with PM from malaria-endemic region of Papua, Indonesia. MtDNA-CN and RTL were determined in 50 placentas by quantitative real-time PCR. Increased placental mtDNA-CN was associated with reduced BW (coef = −193.71, $p = 0.016$), particularly in preterm group (coef = −374.21, $p < 0.001$). RTL did not associate with BW. Increased placental mtDNA-CN indicates a compensatory mechanism to reduced BW in women with PM.

1. Introduction

Placental *Plasmodium falciparum* (*P. falciparum*) malaria (PM), characterized by infected-erythrocytes sequestration and immune cells infiltration in the placenta, causes placental insufficiency and intrauterine growth restriction (IUGR) [1]. PM's adverse effects on birth weight (BW) reduction were mostly reported from highly malaria-endemic Africa [2]. Report from other malaria-endemic regions including Papua, Indonesia, with highly prevalent antimalarial drug-resistance, is still limited [3].

Placenta serves myriad functions to support fetal growth and survival, including facilitating nutrient transport and hormones secretion. Placental activities depend upon ATP generated through mitochondrial oxidative phosphorylation (OXPHOS) [4]. Restricted nutrients, oxidative stress and inflammation may impair mitochondrial OXPHOS, leading to reduced ATP and excessive reactive oxygen species (ROS) productions [4,5]. To compensate for defective mitochondrial functions, placenta undergoes metabolic adaptation by promoting mitochondrial biogenesis, reflected by increased mitochondrial DNA copy number (mtDNA-CN), to fulfill energy demand required for pregnancy maintenance [4]. Furthermore, elevated mitochondrial ROS production contributes to telomere shortening [6]. Elevated mtDNA-CN [7] and shorter telomere [8] in the placenta have been associated with reduced BW, suggesting they may serve as biomarkers for placental insufficiency.

Placental mtDNA-CN and RTL have not been evaluated in PM pregnancy. We therefore assessed their associations with reduced BW in women with PM from Timika, Papua. The evidence will provide valuable insights into the etiology of malaria-associated low BW (LBW).

2. Materials and methods

Fifty RNAlater®-preserved (Invitrogen, Carlsbad, USA), −80°C-stored placentas from *P. falciparum*-infected women in Timika (Papua, Indonesia) were employed (approved by Eijkman Institute Research Ethics Commission (EIREC), No. 19/2005). This study was approved by EIREC, No. 103/2017. Placental tissue assessment, baseline data collection and malaria diagnosis were described elsewhere [9]. Subjects had complete information on age, gravidity, peripheral parasitemia, gestational age, placental weight and newborn's BW, except for haemoglobin (Hb) concentration (n = 46) and axillary temperature (n = 47).

Genomic DNA was extracted using QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany). MtDNA-CN and RTL were determined by quantitative real-time PCR, using SYBR Select MasterMix detection (Applied Biosystem, Foster City, USA) in the 7500 Real-Time PCR System (Applied Biosystem). Primers for mtDNA-CN and RTL detections are described in Supplementary-Table 1. MtDNA-CN measurement was

* Corresponding author.
E-mail address: ina@eijkman.go.id (S.G. Malik).

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Table 1
Result from stepwise linear regression model for variables associated with birth weight (N = 50).

Variable	Adjusted regression coefficient for BW (g)			
	Full Model		Reduced Model	
	Coef	*p*	Coef	*p*
(constant)	773.92	0.781	1247.06	0.637
Log_e mtDNA-CN	−204.78	**0.010**	−193.71	**0.016**
Log_e RTL	−21.90	0.752	–	–
Age (years)	16.87	0.113	13.99	**0.032**
Gravidity (number of pregnancy)	−14.98	0.618	–	–
Peripheral parasitemia (parasite/μL)	< 0.01	0.850	–	–
Hemoglobin concentration (mg/dL)	30.66	0.218	–	–
Axillary temperature (°C)	−99.93	0.154	−111.10	0.098
Gestational age (weeks)	134.43	< **0.001**	142.80	< **0.001**
Placental weight (g)	1.44	< **0.001**	1.29	< **0.001**

Abbreviations: BW, birth weight; Coef, regression coefficient; MtDNA-CN, mitochondrial DNA copy number; RTL, relative telomere length. Multivariate regression model was analysed using ranked-based linear regression. Full model was adjusted for age, gravidity, peripheral parasitemia, hemoglobin concentration, axillary temperature, placental weight and gestational age. Reduced model was adjusted for contributive variables selected by performing the stepwise regression with critical *p* value of 0.15. Significant *p* values are indicated in bold ($p < 0.050$).

as described in Venegas et al. [10]. Telomere repeats were detected using primers from O'Callaghan et al. [11], followed by normalization to the reference gene [10]. MtDNA-CN and RTL were calculated based on the efficiency-corrected method as implemented in "qpcR" package [12].

Data analyses were carried out in R version 3.4.3 (http://www.r-project.org). Missing values (Hb concentration n = 4, axillary temperature n = 3) were accommodated by multiple imputation using random forest (RF) algorithm in the "missForest" package, by training an RF on observed values for each variable, continued by predicting the missing values and proceeding iteratively [13]. Prior to analysis, mtDNA-CN and RTL values were log_e-transformed to reduce skewness. Since the dependent variables are not normally distributed, analyses were performed using ranked-based linear regression [14]. Selection of contributive variables for mtDNA-CN, RTL and BW were performed using stepwise linear regression model with critical *p* value of 0.15. The *p* value of 0.05 was considered significant.

3. Results and discussions

Subject characteristics are described in Supplementary-Table 2. In stepwise regression model, axillary temperature was positively related to placental mtDNA-CN (coef = 0.22, $p = 0.018$) and RTL (coef = 0.30, $p = 0.028$) (Supplementary-Table 3). Raised axillary temperature is typically associated with higher pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) production [15]. These associations suggest the plausible connection between inflammation and mitochondrial biogenesis and telomere modulation. Inflammatory mediators promote mitochondrial biogenesis to compensate for mitochondrial damage and concomitant increased energy demand associated with inflammation [5]. A positive association of axillary temperature to RTL demonstrates a promoting effect of inflammatory responses, like elevated TNF-α, in telomere modulation [16].

Stepwise regression model showed one log_e higher maternal mtDNA-CN was associated with 193.71 g decrease in BW ($p = 0.016$), controlling for age, axillary temperature, gestational age and placental weight (Table 1, Fig. 1A). Significant association was particularly found in the preterm group (coef = −374.21, $p < 0.001$) (Fig. 1B), possibly due to the majority of LBW newborns were born prematurely (14%) (Supplementary-Table 2). The possible influence of IUGR was unclarified, since we did not perform the comprehensive measurement of fetal biometry and growth velocity needed for accurate IUGR diagnosis. An inverse relationship between placental mtDNA-CN and BW was in line with previous reports in IUGR placentas [7] and maternal circulation [17]. Increased mtDNA-CN indicates a placental compensatory response to suboptimal fetal growth under perturbed environment caused by PM, including oxidative stress [18] and placental insufficiency [1]. This response aims to improve mitochondrial bioenergetic capacity to meet energy demand [19], although it also leads to excess ROS production and increase IUGR risk [20].

Placental RTL was not a significant predictor for BW (coef = −21.90, $p = 0.752$) (Table 1), in contrast to a previous report in IUGR pregnancy [8], although tend to decrease with advancing gestational age (coef = −0.09, $p = 0.052$ in reduced model) (Supplementary-Table 3), supporting that telomere shortening plays a role in placental senescence [21]. A study in birds reported telomere accelerated shortening in chronic malaria [22]. In human, chronic infection, particularly when associated with intervillositis, was associated with LBW and preterm delivery [23]. Nevertheless, malaria chronicity was unevaluated in this study, thus its influence on placental RTL and BW necessitates further study.

We acknowledged several study limitations: no non-infected placenta, which restricts direct comparison between pregnancies with and without PM; dietary intake assessment was unevaluated in this study, although it may affect BW, mitochondrial biogenesis and telomere

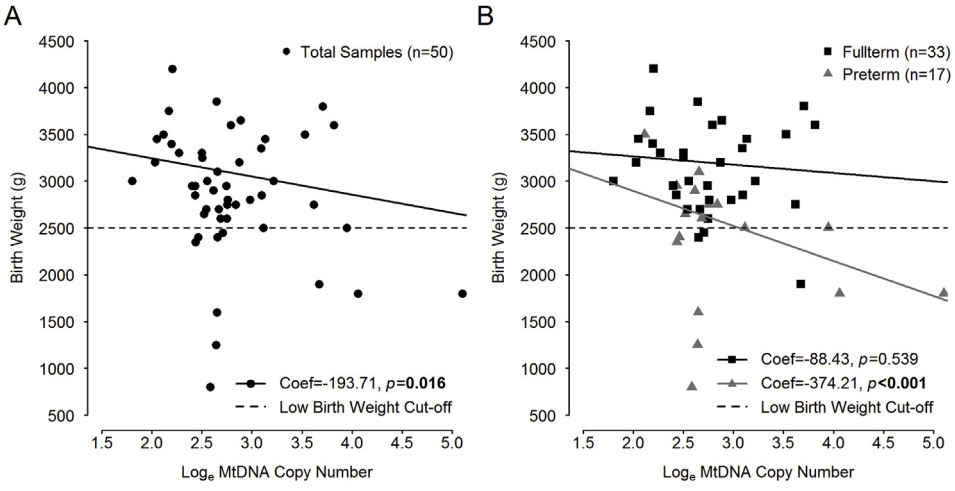

Fig. 1. Association of birth weight (BW) with placental mitochondrial DNA copy number (mtDNA-CN) in total samples (A) and in fullterm and preterm groups (B). Total samples: coef = −193,71, $p = 0.016$, n = 50. Fullterm group: coef = −88.43, $p = 0.539$, n = 33. Preterm group: coef = −374.21, $p < 0.001$, n = 17. Coefficient and *p* value were obtained from rank-based linear regression model, while adjusting for age, axillary temperature, gestational age and placental weight.

attrition [19,24]; besides Hb concentration, this study did not include other maternal nutritional status, like body mass index, height and mid-upper arm circumference [25]. Although malaria infection and malnutrition have been suggested to act independently in increasing LBW risk [2], the interplay between these factors on reduced BW in Papua warrants further study. Thus, our findings should be interpreted cautiously and limited to PM-case only.

In conclusion, increased placental mtDNA-CN is associated with reduced BW in women with PM in Papua, particularly in preterm group. This finding indicates a placental compensatory mechanism to sub-optimal growth and provides further insights into LBW patho-mechanism in pregnancy with PM.

Conflict of interest and funding disclosure

We declare no conflict of interest.

Author contributions

Study idea and design: SGM, RN. Performing the experiments, initial data collection: SO, MF, RN, LT. Data analyses: SO, MF, HT, SGM. Writing and revising the paper: SO, MF, LT, HT, AB, RN, SGM.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.03.005.

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