Original Article

Placental weight ratio affects placental mRNA expression of insulin-like growth factor-I and long isoform of the leptin receptor in *Plasmodium falciparum*-infected pregnant women

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Background and Objectives: *Plasmodium falciparum* infection during pregnancy is characterised by placental inefficiency caused by infected erythrocyte sequestration. Reduced placental efficiency leads to placental intrauterine adaptation for sustaining fetal growth, which is reflected by changes in the expression of placental genes involved in intrauterine growth regulation. Therefore, we aimed to determine whether the placental weight ratio, an indicator of placental efficiency, affects the placental expression of the components of the insulin-like growth factor axis and leptin signalling pathway in *P. falciparum*-infected pregnant women. **Methods and Study Design:** A malaria case-only analysis of 50 *P. falciparum*-infected pregnant women in Timika, Papua, Indonesia, was conducted. The placental mRNA expression of insulin-like growth factor-I, insulin-like growth factor binding protein-1, leptin, and the long and short isoforms of the leptin receptor was measured through quantitative real-time PCR. **Results:** The placental weight ratio exerted a positive effect on the placental mRNA expression of insulin-like growth factor-I (coefficient=6.10, p=0.002) and the long isoform of the leptin receptor (coefficient=4.73, p=0.015) in malaria-infected pregnant women without fever or chill symptoms. **Conclusion:** Our results indicate that placental adaptive responses caused by adverse intrauterine conditions in *P. falciparum*-infected pregnant women vary depending on the presence or absence of fever and chill symptoms.

Key Words: PWR, IGF-I, OBRb, placenta, malaria

INTRODUCTION

Plasmodium falciparum infection is a major health burden for pregnant women living in malaria-endemic regions.[1] The clinical presentations of pregnancy-associated malaria range from asymptomatic to mild symptoms. Furthermore, several parasitised erythrocytes were reported to be sequestered in the placental intervillous space, increasing the risk of low-birth-weight deliveries.[2] Although the pathomechanism of *P. falciparum*-mediated suboptimal growth has not been completely determined, impairment of placental functions caused by parasitic sequestration is a major contributing factor.[3] Studies have supported this notion by implicating several factors that may affect the placental capacity of nutrient transport to the fetus, including placental histological changes (such as thickening of the trophoblastic basal membrane and an increase in the syncytial knots),[4] a reduced placental weight,[5] impaired uteroplacental blood flow,[6] and altered nutrient transport.[7]

Fetal growth is determined by placental growth and the capacity to transport adequate oxygenated blood and nutrients to the fetus.[8] The placental weight ratio (PWR), the placental weight relative to the birth weight, reflects the proportional growth of the placenta and fetus throughout gestation[9] and has been used as an indicator of placental efficiency.[10] During a normal pregnancy, the PWR gradually decreases with advancing gestational age.[9] A high PWR was reported in a pregnancy with intrauterine growth restriction complications.[11] Malaria-associated pregnancies have also been associated with a high PWR,[12] reflecting placental insufficiency, partly because of altered placental nutrient transport.[7]

The placenta acts as an endocrine organ by producing several growth factors and metabolic hormones, including members of the insulin-like growth factor (IGF) axis, for regulating placental and fetal development.[8] IGF-I and IGF binding protein (IGFBP)-1, the essential components of the IGF axis, are crucial for modulating placental and fetal growth. Placental IGF-I stimulates trophoblast pro-

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Manuscript received 24 October 2016. Initial review completed and revision accepted 12 December 2016.
doi: 10.6133/apjcn.122016.s12

liferation and differentiation, trophoblast invasion into the uterine wall, and placental nutrient transport.[13] IGF-I activity is modulated through multiple functions of IGFBP-1, which is mainly produced by the maternal decidua.[14] IGFBP-1 not only maintains the local IGF-I concentrations in various tissues but also inhibits IGF-I activity by binding to free IGF-I proteins because IGFBP-1 has a higher affinity for IGF-I than IGF-I has for its receptor, IGF-1R.[15]

Furthermore, placental tissue expresses leptin and leptin receptors, which have paracrine and autocrine functions.[16] Most of the placental leptin is secreted into the maternal circulation and is involved in regulating maternal appetite, energy metabolism, and fat deposition.[17] In addition, placental leptin influences placental growth and development by stimulating trophoblast proliferation and nutrient transport and regulating placental angiogenesis and immune responses.[16] Leptin exerts its biological actions by binding to its long and short receptors, which are expressed in the placental tissues.[18] The long isoform of the leptin receptor (OBRb) mediates multiple leptin-stimulated signalling pathways,[19] whereas the short isoform (OBRa) is less crucial for cellular signalling and is more involved in transplacental leptin transport.[20]

Studies have reported that IGF-I and leptin expression did not differ significantly between pregnancies with and those without malaria infection.[21,22] However, whether placental inefficiency caused by adverse intrauterine conditions associated with malarial parasitic sequestration influences the placental expression of these genes remains unknown.

This study aimed to investigate the effect of the PWR, an indicator of placental efficiency, on the placental mRNA expression of IGF-I, IGFBP-1, leptin, and leptin receptors in *P. falciparum*-infected pregnant women. Information on placental hormone mRNA expression profiles will provide new insights for understanding the molecular mechanisms that influence optimal fetal growth in pregnancies complicated by *P. falciparum* infection. Furthermore, maternal and perinatal health can be improved and more appropriate prevention strategies can be developed using the aforementioned information.

MATERIALS AND METHODS

Samples

In this study, we utilised 50 archived placental tissue samples, which were collected during a study (unpublished data) funded by the World Health Organisation (study number A41399 to Noviyanti R.) and approved by the Eijkman Institute Research Ethics Commission (number 19/2005). In the aforementioned study, informed consent was obtained from participants with singleton pregnancies and *P. falciparum* infection at Rumah Sakit Mitra Masyarakat, Timika, Papua, Indonesia, from 2005 to 2008. Furthermore, the present study was approved by the Eijkman Institute Research Ethics Commission (number 43/2010).

The pregnant women were recruited after *P. falciparum* parasitaemia was diagnosed on microscopic examination of their peripheral blood smears at the delivery unit prior to delivery. Peripheral parasitaemia was estimated by counting the number of parasites per 200 white blood cells in a thick blood smear, assuming a total white blood cell count of 8000 cells/μL. Furthermore, *P. falciparum* infection was confirmed using nested PCR assays that targeted the parasite's small-subunit ribosomal RNA gene.[23]

The unwashed placentae (without umbilical cords) were weighed using a digital scale immediately after delivery. Placental biopsy specimens were collected from the maternal side of the placentae. A small placental biopsy specimen (approximately 0.5 cm in each dimension) was stored in a screw-top tube containing RNA*later*® (Ambion, Foster City, CA, USA). The presence of *P. falciparum* infections in the placentae was determined using the nested PCR assays as previously described.[23]

Maternal data, including age, gravidity, and history of fever and/or chills during pregnancies (fever prior to delivery or a previous history of fever and/or chill symptoms during pregnancy) were collected upon recruitment. Newborns were weighed immediately after delivery using a digital scale, and their gestational age was estimated according to the first day of the last menstrual period and the fundal height measurement. The PWR was defined as the placental weight divided by the birth weight as previously described.[9]

RNA extraction

Total RNA was extracted from the placental tissues stabilised in RNA*later*® (Ambion) and stored at −80°C. In brief, approximately 10 mg of the placental tissue was ground using a mortar and pestle precooled in liquid nitrogen. The powdered tissue was added to the RLT lysis buffer obtained from the RNeasy minikit (Qiagen, Hilden, Germany) and homogenised using a QIAshredder spin column (Qiagen). Total RNA extraction from tissue lysates was performed using the RNeasy minikit (Qiagen) according to the manufacturer's instructions. Genomic DNA was extracted using the Turbo DNA-free kit (Applied Biosystems, Foster City, CA, USA), and removal was confirmed by an absent amplification signal following quantitative real-time PCR (qPCR). The RNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm, respectively, using a NanoDrop-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Complementary DNA synthesis

Complementary DNA (cDNA) synthesis was performed using high-capacity cDNA reverse transcription kits with RNase inhibitors (Applied Biosystems) according to the manufacturer's instructions. The isolated RNA (1.5 g) was added to a 20 μL reaction mixture (Applied Biosystems) containing 1x reverse transcription buffer, 1x random primer, 1x deoxynucleotide mix, 50 U of MultiScribe™ reverse transcriptase, and 20 U of RNase inhibitor. Reverse transcription was performed using a thermal cycler machine, GeneAmp® PCR System 9700 (Applied Biosystems), under the following conditions: 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. The cDNA products were stored at −20°C until use.

Quantitative real-time PCR

The relative mRNA expression levels of IGF-I, IGFBP-1,

leptin, OBRb, and OBRa were determined through qPCR using TaqMan hydrolysis probes (Applied Biosystems). Inventoried TaqMan gene expression assays were used for detecting IGF-I (Hs01547656_m1), IGFBP-1 (Hs00236877_m1), leptin (Hs00174877_m1), OBRb (Hs00174492_m1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1) transcripts. A customised TaqMan gene expression assay with primer and probe sequences was used to detect the OBRa transcript as previously described.[24] qPCR was performed in duplicate using a 20 μL reaction mixture consisting of 40 ng of the cDNA template, 1x TaqMan Universal PCR Master Mix, 1x TaqMan Gene Expression Assay probes and primers (or 500 nM primers and 200 nM probes for the OBRa transcript). Because of the high abundance of the reference gene transcript, qPCR for GAPDH was performed using a low quantity of the cDNA template (1 ng) for obtaining quantification cycle (Cq) values within the range of target gene Cq values (data not shown). A control containing deionized RNase-free water instead of the cDNA template was included in each qPCR. Reactions were monitored using the 7500 Real-Time PCR System (Applied Biosystems) under the following conditions: one cycle at 50°C for 2 minutes and one cycle of predenaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation (95°C for 15 seconds), and the combined annealing/elongation (60°C for 1 minute). Raw fluorescence data (dRn) were exported as text files and analysed using the qPCR package from the R Project for Statistical Computing version 3.2.2 (http://www.r-project.org). The Cq values and amplification efficiency were calculated from the second derivative maximum (CpD2) of each amplification curve using a dRn-fitted five-parameter log-logistic model.[25] The relative expression ratio of each gene was calculated using the average Cq and amplification efficiency values of the duplicates and was normalised to the reference GAPDH transcript according to the efficiency-corrected method by Pfaffl et al.[26] A calibration curve was constructed for each qPCR assay using 2-fold serial dilutions of the cDNA template in duplicate. Reference gene stability was evaluated using the BestKeeper software as previously described.[27]

Data analysis

Data analysis was performed with the R Project for Statistical Computing (version 3.2.2) by using custom scripts from the stats and R-fit packages. Normality of continuous variables was assessed using the Shapiro–Wilk test. Data on the placental mRNA expression and PWR were log-transformed. Because most of the continuous variables were not normally distributed, data were presented as the median (first quartile–third quartile) and rank-based linear regression analysis, which does not require the assumption of normality, was performed.[28] Univariate linear regression analysis was performed for determining the association between the pregnancy parameters (maternal age, gravidity, peripheral parasitaemia, gestational age, fever and/or chill symptoms, and PWR) and the placental mRNA expression. In addition, multivariate linear regression analyses were performed for investigating the influence of the PWR on the placental mRNA expression after adjustment for potential confounders. Gestational age and peripheral parasitaemia were associated with placental mRNA expression in univariate analysis; therefore, these factors were included as covariates in the multivariate model. In addition, maternal age, gravidity, and fever and/or chill symptoms were included as covariates in the multivariate model, regardless of their p values. For investigating the role of fever and/or chill symptoms, multivariate regression analyses of the placental mRNA expression with the PWR (adjusted for maternal age, gravidity, peripheral parasitaemia, and gestational age) were performed for malaria-infected women without (N=20) and with (N=30) fever and/or chill symptoms during their pregnancies. Values of $p<0.05$ were considered statistically significant.

RESULTS

Fifty pregnant women with *P. falciparum* infections in their peripheral blood and placentae were recruited in this study. Characteristics of the study participants are presented in Table 1. The median age and gravidity of the participants were 22.0 years (18.0–28.0 years) and 2.00 (1.00–3.00), respectively. The median peripheral parasitaemia level was 4780 parasites/μL (740–31640 parasites/μL). Approximately 60% of these patients reported fever and/or chills during their pregnancies. Newborns had a median gestational age, placental weight, birth weight, and PWR of 38.0 weeks (36.5–38.5 weeks), 570 g (500–635 g), 2925 g (2600–3338 g), and 0.20 (0.17–0.22), respectively.

In this study, the placental mRNA expression was measured using the placental tissues collected from the maternal side of the placentae, where the malaria-infected erythrocytes were sequestered. The qPCR conducted for measuring the placental mRNA expression was validated using the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines.[29] The qPCR assay amplification efficiencies for GAPDH, IGF-I, IGFBP-1, leptin, OBRb, and OBRa were 1.46, 1.63, 1.52, 1.60, 1.74, and 1.69, respectively. The coefficients of determination (R^2) for all calibration curves exhibited a strong linear relationship (>0.99) between the cDNA template concentrations and Cq values (data not shown).

Table 1. Characteristics of subjects (N=50)

Characteristics	Observation
Mothers	
Maternal age (years)	22.0 (18.0-28.0)
Gravidity (number of pregnancy)	2.00 (1.00-3.00)
Peripheral parasitemia (parasites/μl)	4780 (740-31640)
History of fever/chills at anytime during pregnancy [n (%)]	
No fever/chills	20 (40)
Fever/chills	30 (60)
Newborns	
Gestational age (weeks)	38.0 (36.5-38.5)
Placental weight (g)	570 (500-635)
Birth weight (g)	2925 (2600-3338)
Placental weight ratio (PWR)	0.20 (0.17-0.22)

Continuous variables are presented as median (1st quartile-3rd quartile). Categorical variables are presented as number of observation (%).

Table 2. Univariate regression results of placental mRNA expressions and pregnancy parameters

Variables	Log IGF-I		Log IGFBP-1		Log Leptin		Log OBRb		Log OBRa	
	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*
Age	0.02	0.665	0.05	0.430	-0.02	0.756	-0.05	0.258	-0.05	0.344
Gravidity	0.13	0.305	-0.09	0.649	0.01	0.932	-0.12	0.282	-0.12	0.439
Peripheral parasitemia	0.00	0.672	0.00	0.035*	0.00	0.102	0.00	0.157	0.00	0.095
Gestational age	-0.18	0.095	-0.27	0.129	-0.06	0.630	-0.19	0.080	-0.31	0.045*
Fever/chills	0.62	0.286	-1.49	0.160	-0.06	0.935	0.90	0.155	1.03	0.309
Log PWR	12.0	0.028*	5.71	0.523	8.88	0.213	6.89	0.326	10.45	0.265

PWR: placental weight ratio; Coef: coefficient of regression; IGF-I: insulin-like growth factor-I; IGFBP-1: insulin-like growth factor binding protein-1; OBRb: long isoform of the leptin receptor; OBRa: short isoform of the leptin receptor.
*Significant *p* values of Wald's test ($p<0.05$).

Table 3. Multivariate regression analyses for placental mRNA expressions

Variables	Log IGF-I		Log IGFBP-1		Log Leptin		Log OBRb		Log OBRa	
	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*
Log PWR	2.20	0.134	0.66	0.735	1.61	0.395	0.22	0.884	-0.56	0.797
Age	-0.04	0.501	0.22	0.022*	0.01	0.958	-0.03	0.667	-0.08	0.448
Gravidity	0.27	0.501	-0.38	0.165	0.07	0.793	-0.01	0.963	0.11	0.719
Peripheral parasitemia	-0.00	0.763	0.00	0.006*	0.00	0.072	0.00	0.693	0.00	0.486
Gestational age	-0.16	0.184	-0.08	0.610	0.08	0.601	-0.12	0.339	-0.29	0.113
Fever/chills	0.08	0.897	-1.83	0.044*	-0.85	0.324	0.88	0.210	0.80	0.424

PWR: placental weight ratio; Coef: coefficient of regression; IGF-I: insulin-like growth factor-I; IGFBP-1: insulin-like growth factor binding protein-1; OBRb: long isoform of the leptin receptor; OBRa: short isoform of the leptin receptor.
The model used for regression was: Log mRNA expression ~ log PWR + age + gravidity + peripheral parasitemia + gestational age + fever/chills symptoms.
*Significant *p* values of Wald's test ($p<0.05$).

Analysis of reference gene stability revealed a standard deviation (SD), coefficient of variation (CV), and maximum fold change (MFC) of 0.91, 2.94%, and 1.94, respectively, confirming the stability of the reference gene (SD <1, CV <4%, MFC <2).[27]

Table 2 summarises the univariate regressions between placental mRNA expression and pregnancy parameters. Higher placental IGF-I mRNA expression was associated with an increased PWR (coefficient=12.0, p=0.028). Moreover, higher placental IGFBP-1 mRNA expression was significantly associated with higher peripheral parasitaemia (coefficient <0.01, p=0.035). Furthermore, peripheral parasitaemia and OBRa expression exhibited a non-significant positive association (coefficient <0.01, p=0.095). Higher placental OBRa mRNA expression was associated with reduced gestational age (coefficient=−0.31, p=0.045). A similar trend was observed for placental IGF-I and OBRb mRNA expression; however, these associations were not statistically significant (IGF-I: coefficient=−0.18, p=0.095; OBRa: coefficient=−0.19, p=0.080).

Furthermore, for investigating the influence of the PWR on the placental mRNA expression, multivariate regression analyses were performed after adjustment for the potential confounders (Table 3). Peripheral parasitaemia and gestational age were significantly associated with placental mRNA expression (Table 2); therefore, these factors were included as covariates in the regression models, whereas maternal age, gravidity, and fever and/or chill symptoms were included in these models, regardless of their p values. After adjustment for confounders, none of these genes were significantly affected by the PWR.

The study participants had varying clinical characteristics; therefore, to ascertain whether fever and/or chill symptoms influence the effects of the PWR on placental mRNA expression, multivariate regression analyses were conducted for cases of malaria infection during pregnancies with and without fever and/or chill symptoms (Table 4). A significant positive effect of the PWR on the placental expression of IGF-I and OBRb transcripts was observed in pregnancies without fever or chill symptoms (IGF-I: coefficient=6.10, p=0.002; OBRb: coefficient=4.73, p=0.015) but not in pregnancies with fever and/or chill symptoms (IGF-I: coefficient=−1.25, p=0.437; OBRb: coefficient=2.02, p=0.234, respectively).

DISCUSSION

Compared with pregnancies without infection, those with *P. falciparum* infection exhibit reduced birth and placental weights.[2,5] The placenta is crucial for supporting fetal growth; therefore, the PWR has been considered as an indicator of placental efficiency in transporting nutrients and oxygenated blood to support fetal growth.[11] Furthermore, we observed that placental IGF-I and OBRb mRNA expression in pregnancies with *P. falciparum* infection was positively influenced by the PWR, particularly in the absence of fever or chill symptoms during pregnancy.

The placenta is crucial for maintaining fetal growth through metabolic adaptation to maternal nutritional availability for satisfying the fetal nutrient demand.[8] Decreased placental efficiency because of altered amino acid transport between maternal and fetal circulations was reported in malaria-infected pregnant women, particularly in association with intervillositis.[7] Furthermore, placental malaria with intervillositis was associated with a decrease

Table 4. Multivariate regression analyses for placental mRNA expressions in malaria-infected pregnancies without (N=20) and with (N=30) fever/chills symptoms

Symptoms	Variable	Log IGF-I		Log IGFBP-1		Log Leptin		Log OBRb		Log OBRa	
		Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*	Coef.	*p*
No-symptoms	Log PWR	6.10	0.002*	2.42	0.454	-0.81	0.783	4.73	0.015*	4.47	0.159
	Age	0.05	0.598	0.36	0.064	-0.22	0.199	0.11	0.252	0.34	0.067
	Gravidity	-0.23	0.390	-0.98	0.066	0.53	0.265	-0.39	0.171	-0.90	0.077
	Peripheral parasitemia	-0.00	0.021*	0.00	0.453	-0.00	0.679	-0.00	0.023*	-0.00	0.050
	Gestational age	-0.23	0.227	0.36	0.920	0.02	0.962	-0.38	0.070	-0.48	0.184
Fever/chills	Log PWR	-1.25	0.437	-1.90	0.494	1.83	0.592	2.02	0.234	-0.13	0.956
	Age	-0.09	0.228	0.01	0.926	0.16	0.304	-0.12	0.115	-0.19	0.094
	Gravidity	0.38	0.069	0.16	0.652	-0.31	0.471	0.23	0.289	0.46	0.156
	Peripheral parasitemia	-0.00	0.826	0.00	0.064	0.00	0.079	0.00	0.275	0.00	0.226
	Gestational age	-0.25	0.039*	0.27	0.178	0.15	0.537	-0.13	0.281	-0.15	0.402

PWR: placental weight ratio; Coef: coefficient of regression; IGF-I: insulin-like growth factor-I; IGFBP-1: insulin-like growth factor binding protein-1; OBRb: long isoform of the leptin receptor; OBRa: short isoform of the leptin receptor.
The model used for regression was: Log mRNA expression ~ log PWR + age + gravidity + peripheral parasitemia + gestational age.
*Significant *p* values of Wald's test ($p<0.05$).

in placental glucose transporter (GLUT-1) expression.[30] Altered nutrient transport associated with malaria infection during pregnancy may lead to intrauterine malnutrition, consequently triggering placental adaptive responses, which are reflected by the changes in the expression of placental genes regulating fetal growth.

A study reported that placental IGF-I gene expression did not differ significantly in malaria-infected and uninfected pregnant women with and without intervillositis.[21] In this malaria case-only study, we observed that an increase in the PWR resulted in increased placental IGF-I mRNA expression, particularly in malaria-infected pregnant women without fever or chill symptoms. IGF-I promotes placental nutrient transport by inducing placental GLUT-1 expression[31] and stimulating the uptake of glucose and amino acids.[32,33] A study reported that placental IGF-I expression is modulated by nutritional stimuli; the placental IGF-I mRNA expression was higher in an animal model provided with a low protein diet to compensate for the reduced nutrient availability.[34] A high PWR indicates low placental functional efficiency[9]; therefore, the positive effect of the PWR on the placental IGF-I mRNA expression observed in this study may indicate the presence of a placental adaptive response for enhancing nutrient transport for fetal growth. The lack of a PWR influence on the placental IGF-I mRNA expression in pregnancies with fever and/or chill symptoms suggested that other factors related to malarial symptoms modulate placental gene expression. Some possible modulators, namely proinflammatory cytokines including tumour necrosis factor (TNF)-α and interferon-γ, exhibited increased levels in pregnancies with *P. falciparum* infection and were associated with malaria-related fever and impaired fetal growth.[35] Furthermore, these proinflammatory cytokines reduce IGF-I transcription by inhibiting the growth hormone-mediated signalling pathway.[36]

Among the mRNA transcripts involved in leptin signalling in the placenta, only the placental OBRb mRNA expression was associated with the PWR in patients without fever or chill symptoms during their pregnancies. OBRb is the only isoform of the leptin receptor that can execute leptin-activated signal transduction.[19] Furthermore, a study reported that placental OBRb mRNA expression was downregulated in protein-deficient rats,[34] suggesting that it could be modulated by the maternal nutritional status during pregnancy. Therefore, the positive effect of the PWR on placental OBRb mRNA expression demonstrated that this receptor isoform is crucial for promoting leptin signalling in the placenta. Furthermore, absence of a PWR-mediated increase in OBRb expression in patients with fever and/or chill symptoms may indicate that the fever-associated inflammatory conditions conceal the effect of the PWR on the OBRb expression; this finding is consistent with a study that revealed that TNF-α upregulates OBRb expression.[37] Although not statistically significant, the placental OBRb expression was higher in patients with fever and/or chill symptoms (data not shown).

This study indicated an association between higher peripheral parasitaemia and increased placental IGFBP-1 and OBRa mRNA expression in univariate analysis, suggesting an adaptive placental response to systemic inflammation caused by high parasite density in the maternal circulation.[38] Furthermore, studies have reported that increased levels of proinflammatory cytokines, such as interleukin (IL)-6, IL-1, and TNF-α, were associated with increased IGFBP-1 expression.[39,40] Although the underlying regulatory mechanisms governing OBRa expression remain unclear, this isoform is upregulated under inflammatory conditions in the peritoneal adipose tissue of diabetic mice.[41] These findings indicate that placental leptin receptor isoforms are differentially regulated in response to adverse intrauterine conditions associated with malaria and warrant further investigation.

The adverse intrauterine conditions triggering fetal adaptation, including placental insufficiency, are associated with an increased risk of chronic noncommunicable diseases in adulthood.[42] Disproportionate growth of the placenta relative to the fetus indicates a placental intrauterine adaptive response, in which the placental growth is accelerated to compensate for the altered placental capacity because of adverse intrauterine conditions. Therefore, the

placenta continues to provide an adequate nutrient supply to the growing fetus. These results revealed that the PWR influences mRNA expression-mediated fetal–placental growth in the absence of fever or chill symptoms, indicating a placental adaptation mechanism for sustaining fetal growth. The reduced effect of the PWR in patients with fever and/or chill symptoms may be attributed to increased levels of proinflammatory mediators, which influence the placental gene expression and mask the effect of the PWR on mRNA expression of the components of the IGF axis and leptin signalling pathway. However, the lack of normal placentae as controls was a limitation that prevented the present study from directly comparing the pathogenesis of suboptimal growth between normal pregnancies and those with malaria infection. Nevertheless, the different trends observed between patients with and without fever and/or chill symptoms warrant further investigation into the roles of inflammatory and growth signals involved in placental responses to the numerous malarial clinical presentations.

In addition, we did not directly measure the functional protein. Although the mRNA levels do not always correlate with the protein levels, the mRNA levels are typically an exceptional indicator of protein presence.[43] Furthermore, several studies have reported similar patterns of mRNA and protein expression for the components of the IGF axis and leptin signalling pathway.[44–46]

Nutritional deprivation remains an unsolved problem in Timika. The 2007 National Basic Health Research report (Riset Kesehatan Dasar/Riskesdas 2007) revealed that the average energy consumed in the Mimika District (where Timika is located) was lower than the national average in 2007 (1202.1 vs 1735.5 kcal, respectively).[47] Whether maternal nutritional status modulates the placental expression of the components of the IGF axis and leptin signalling pathway was not addressed in this study. The iron status has been reported to modify the risk of malaria and improve birth outcomes.[48] According to report on health profile of Indonesia in 2014, the iron supplementation coverage of pregnant woman in the Papua Province was only 49.1%.[49] These data raise a possible interaction between iron status and malaria in contributing the risk of suboptimal fetal growth. However, the iron status and iron supplementation data of *P. faciparum*-infected pregnant women were not available in the study. Further research is warranted for identifying the underlying mechanisms leading to placental gene expression changes, particularly in malaria-infected pregnancies causing impaired fetal growth.

Conclusions

The PWR positively influenced placental IGF-I and OBRb mRNA expression in pregnancies without fever or chill symptoms. These results indicated that atypical placental adaptive responses may compensate for adverse intrauterine conditions that vary depending on the presence or absence of clinical malarial symptoms. Placental adaptation in pregnancies with malaria infection exerts a potential long-term health effect on pregnant women and their children living in malaria-endemic regions, emphasising the importance of the prevention and control of malaria transmission.

ACKNOWLEDGEMENTS

The authors are grateful to the study participants. The authors acknowledge Professor Stephen Rogerson, Professor Inge Sutanto, Dr. Yuditiya Purwosunu, and Dr. Titis Prawitasari for helpful suggestions and comments during the preparation of this manuscript. The authors also thank Retno Ayu Setya Utami and Agatha Mia Puspitasari for technical help in performing nested PCR assays to detect *P. falciparum* infections.

AUTHOR DISCLOSURES

The authors declare that they have no competing interests. This study was supported by the Institutional Research Grant from the Danone Institute Indonesia (No. I-016, 2012).

REFERENCES

1. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B et al. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7:93-104. doi: 10.1016/s1473-3099(07)70021-x.
2. Uneke CJ. Impact of placental Plasmodium falciparum malaria on pregnancy and perinatal outcome in sub-Saharan Africa: I: introduction to placental malaria. Yale J Biol Med. 2007;80:39.
3. Rogerson SJ, Mwapasa V, Meshnick SR. Malaria in pregnancy: linking immunity and pathogenesis to prevention. Am J Trop Med Hyg. 2007;77(6 Suppl):14-22.
4. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Hum Pathol. 2000;31:85-93. doi: 10.1016/s0046-8177(00)80203-8.
5. Singh J, Soni D, Mishra D, Singh HP, Bijesh S. Placental and neonatal outcome in maternal malaria. Indian Pediatr. 2014;51:285-8. doi: 10.1007/s13312-014-0402-3.
6. Dorman EK, Shulman CE, Kingdom J, Bulmer JN, Mwendwa J, Peshu N et al. Impaired uteroplacental blood flow in pregnancies complicated by falciparum malaria. Ultrasound Obstet Gynecol. 2002;19:165-70. doi: 10.1046/j.0960-7692.2001.00545.x.
7. Boeuf P, Aitken EH, Chandrasiri U, Chua CLL, McInerney B, McQuade L et al. Plasmodium falciparum malaria elicits inflammatory responses that dysregulate placental amino acid transport. PLoS Pathog. 2013;9:e1003153. doi: 10.1371/journal.ppat.1003153.
8. Myatt L. Placental adaptive responses and fetal programming. J Physiol. 2006;572(Pt 1):25-30. doi: 10.1113/jphysiol.2006.104968.
9. Macdonald EM, Koval JJ, Natale R, Regnault T, Campbell MK. Population-based placental weight ratio distributions. Int J Pediatr. 2014;2014:1-7. doi: 10.1155/2015/181257.
10. Hayward CE, Lean S, Sibley CP, Jones RL, Wareing M, Greenwood SL et al. Placental adaptation: what can we learn from birthweight: placental weight ratio? Front Physiol. 2016;7:28. doi: 10.3389/fphys.2016.00028.
11. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation: placental efficiency and adaptation. J Physiol. 2009;587:3459-72. doi: 10.1113/jphysiol.2009.173013.
12. Brabin BJ, Romagosa C, Abdelgalil S, Menéndez C, Verhoeff FH, McGready R et al. The sick placenta-the role of malaria. Placenta. 2004;25:359-78. doi: 10.1016/j.placenta.2003.10.019.
13. Forbes K, Westwood M. The IGF axis and placental function: a mini review. Horm Res. 2008;69:129-37. doi: 10.1159/000112585.

14. Gibson JM, Aplin JD, White A, Westwood M. Regulation of IGF bioavailability in pregnancy. Mol Hum Reprod. 2001; 7:79-87. doi: 10.1093/molehr/7.1.79.
15. Crossey PA, Pillai CC, Miell JP. Altered placental development and intrauterine growth restriction in IGF binding protein-1 transgenic mice. J Clin Invest. 2002;110:411-8. doi: 10.1172/jci0210077.
16. Ashworth CJ, Hoggard N, Thomas L, Mercer JG, Wallace JM, Lea RG. Placental leptin. Rev Reprod. 2000;5:18-24. doi: 10.1530/revreprod/5.1.18.
17. Linnemann K, Malek A, Sager R, Blum WF, Schneider H, Fusch C. Leptin production and release in the dually in vitro perfused human placenta. J Clin Endocrinol Metab. 2000;85: 4298-301. doi: 10.1210/jc.85.11.4298.
18. Bodner J, Ebenbichler CF, Wolf HJ, Müller-Holzner E, Stanzl U, Gander R et al. Leptin receptor in human term placenta: in situ hybridization and immunohistochemical localization. Placenta. 1999;20:677-82. doi: 10.1053/plac.1999.0431.
19. Tartaglia LA. The leptin receptor. J Biol Chem. 1997;272: 6093-6. doi: 10.1074/jbc.272.10.6093.
20. Smith JT, Waddell BJ. Leptin distribution and metabolism in the pregnant rat: transplacental leptin passage increases in late gestation but is reduced by excess glucocorticoids. Endocrinology. 2003;144:3024-30. doi: 10.1210/en.2003-0145.
21. Umbers AJ, Boeuf P, Clapham C, Stanisic DI, Baiwog F, Mueller I et al. Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation. J Infect Dis. 2011;203:561-9. doi: 10.1093/infdis/jiq080.
22. Kabyemela ER, Fried M, Kurtis JD, Mutabingwa TK, Duffy PE. Fetal responses during placental malaria modify the risk of low birth weight. Infect Immun. 2008;76:1527-34. doi: 10.1128/iai.00964-07.
23. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus-and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg. 1999;60:687-92.
24. Meller M, Qiu C, Kuske BT, Abetew DF, Muy-Rivera M, Williams MA. Adipocytokine expression in placentas from pre-eclamptic and chronic hypertensive patients. Gynecol Endocrinol. 2006;22:267-73. doi: 10.1080/09513590600630421.
25. Spiess A-N, Feig C, Ritz C. Highly accurate sigmoidal fitting of real-time PCR data by introducing a parameter for asymmetry. BMC Bioinformatics. 2008;9:221. doi: 10.1186/1471-2105-9-221.
26. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29: e45. doi: 10.1093/nar/29.9.e45.
27. Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper--Excel-based tool using pair-wise correlations. Biotechnol Lett. 2004;26:509-15. doi: 10.1023/b:bile.0000019559.84305.47.
28. Hettmansperger TP, McKean JW. Robust nonparametric statistical methods. London : New York: Arnold ; J Wiley & Sons; 1998. p. 467.
29. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55:611-22. doi: 10.1373/clinchem.2008.112797.
30. Chandrasiri UP, Chua CLL, Umbers AJ, Chaluluka E, Glazier JD, Rogerson SJ et al. Insight into the pathogenesis of fetal growth restriction in placental malaria: decreased placental glucose transporter isoform 1 expression. J Infect Dis. 2014;209:1663-7. doi: 10.1093/infdis/jit803.
31. Baumann MU, Schneider H, Malek A, Palta V, Surbek DV, Sager R et al. Regulation of human trophoblast GLUT1 glucose transporter by insulin-like growth factor I (IGF-I). PLoS One. 2014;9:e106037. doi: 10.1371/journal.pone.0106037.
32. Jones HN, Crombleholme T, Habli M. Adenoviral-mediated placental gene transfer of IGF-1 corrects placental insufficiency via enhanced placental glucose transport mechanisms. PLoS One. 2013;8:e74632. doi: 10.1371/journal.pone.0074632.
33. Karl PI. Insulin-like growth factor-1 stimulates amino acid uptake by the cultured human placental trophoblast. J Cell Physiol. 1995;165:83-8. doi: 10.1002/jcp.1041650111.
34. Nüsken K-D, Schneider H, Plank C, Trollmann R, Nüsken E, Rascher W et al. Fetal programming of gene expression in growth-restricted rats depends on the cause of low birth weight. Endocrinology. 2011;152:1327-35. doi: 10.1210/en.2010-1116.
35. Rogerson SJ, Brown HC, Pollina E, Abrams ET, Tadesse E, Lema VM et al. Placental tumor necrosis factor alpha but not gamma interferon is associated with placental malaria and low birth weight in Malawian women. Infect Immun. 2003;71:267-70. doi: 10.1128/iai.71.1.267-270.2003.
36. Wolf M, Böhm S, Brand M, Kreymann G. Proinflammatory cytokines interleukin 1β and tumor necrosis factor α inhibit growth hormone stimulation of insulin-like growth factor I synthesis and growth hormone receptor mRNA levels in cultured rat liver cells. Eur J Endocrinol. 1996;135:729-37. doi: 10.1530/eje.0.1350729.
37. Gan L, Guo K, Cremona ML, McGraw TE, Leibel RL, Zhang Y. TNF-α up-regulates protein level and cell surface expression of the leptin receptor by stimulating its export via a PKC-dependent mechanism. Endocrinology. 2012;153: 5821-33. doi: 10.1210/en.2012-1510.
38. Thevenon AD, Zhou JA, Megnekou R, Ako S, Leke RGF, Taylor DW. Elevated levels of soluble TNF receptors 1 and 2 correlate with Plasmodium falciparum parasitemia in pregnant women: potential markers for malaria-associated inflammation. J Immunol. 2010;185:7115-22. doi: 10.4049/jimmunol.1002293
39. Samstein B, Hoimes ML, Fan J, Frost RA, Gelato MC, Lang CH. IL-6 stimulation of insulin-like growth factor binding protein (IGFBP)-1 production. Biochem Biophys Res Commun. 1996;228:611-5. doi: 10.1006/bbrc.1996.1705.
40. Benbassat CA, Lazarus DD, Cichy SB, Evans TM, Moldawer LL, Lowry SF et al. Interleukin-1 alpha (IL-1 alpha) and tumor necrosis factor alpha (TNF alpha) regulate insulin-like growth factor binding protein-1 (IGFBP-1) levels and mRNA abundance in vivo and in vitro. Horm Metab Res. 1999;31:209-15. doi: 10.1055/s-2007-978721.
41. Leung JCK, Chan LYY, Lam MF, Tang SCW, Chow CW, Lim AI et al. The role of leptin and its short-form receptor in inflammation in db/db mice infused with peritoneal dialysis fluid. Nephrol Dial Transplant. 2012;27:3119-29. doi: 10.1093/ndt/gfr774.
42. Moor V, Davies M. Early life influences on later health: the role of nutrition. Asia Pac J Clin Nutr. 2001;10:113-7. doi: 10.1046/j.1440-6047.2001.00237.x
43. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13:227-32. doi: 10.1038/nrg3185.
44. Street ME, Seghini P, Fieni S, Ziveri MA, Volta C, Martorana D et al. Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns

with fetal growth restriction compared with controls. Eur J Endocrinol. 2006;155:567-74. doi: 10.1530/eje.1.02251.
45. Akram SK, Carlsson-Skwirut C, Bhutta ZA, Söder O. Placental IGF-I, IGFBP-1, zinc, and iron, and maternal and infant anthropometry at birth. Acta Paediatr. 2011;100:1504-9. doi: 10.1111/j.1651-2227.2011.02336.x.
46. Tzschoppe A, Struwe E, Rascher W, Dörr HG, Schild RL, Goecke TW et al. Intrauterine growth restriction (IUGR) is associated with increased leptin synthesis and binding capability in neonates. Clin Endocrinol (Oxf). 2011;74:459-66. doi: 10.1111/j.1365-2265.2010.03943.x.
47. The National Institute of Health Research and Development. Report on result of national basic health research (RISK-ESDAS) 2007 in Papua Province. Jakarta: Ministry of Health, Republic of Indonesia; 2008.
48. Sangaré L, van Eijk AM, Ter Kuile FO, Walson J, Stergachis A. The association between malaria and iron status or supplementation in pregnancy: a systematic review and meta-analysis. PLoS One. 2014;9:e87743. doi: 10.1371/journal.pone.0087743.
49. Ministry of Health, Republic of Indonesia. Health profile of Indonesia 2014. Jakarta: Ministry of Health, Republic of Indonesia; 2015.