Impact of placental malaria on maternal, placental and foetal cord responses and its role in pregnancy outcomes in women from Blue Nile State, Sudan

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Abstract

Background The sequestration of *Plasmodium falciparum* infected cells in the placenta results in placental malaria (PM). It activates the mother's immune cells and induces secretion of inflammatory cytokines, which might influence pregnancy outcomes. This study aims to investigate the inflammatory environment in maternal peripheral, placental, and umbilical cord blood in response to PM and the extent to which this may influence maternal haemoglobin levels and birth weight.

Methods A total of 185 consenting Sudanese women from Blue Nile state were enrolled in a cross sectional conducted between Jan 2012-Dec 2005. Malaria infection in the collected samples was determined microscopically, and ELISA was used to measure the plasma levels of the antibodies, IL-4, IL-6, IL-10, IL-17A, and INF γ in the collected positive and negative malaria samples.

Results Elevated levels of antibodies, IL-4 and IL-10 and reduced levels of IL-6 were detected in the malaria positive samples in comparison to the negative ones in the three types of samples investigated. Maternal antibodies, IL-4 and IL-10 were significantly higher in the samples collected from the PM infected group compared to the non-infected control ($P < 0.001$). While the absence of PM was significantly associated with the IL-6 and maternal IFN-γ levels, maternal IL-17A, placental and umbilical cord IFN-γ levels showed no significant difference ($P=0.214$, $P=0.065$, $P=0.536$, respectively) due to infection. Haemoglobin level and birth weight were increased in the group with high levels of IL-6 and IL-17A, but not in the group with IL-4 and IL-10 levels. While significantly negative correlation was found between IFN-γ levels and birth weight for all three types of samples, only maternal peripheral FN-γ level was significantly positively correlated with maternal haemoglobin ($r= 0.171$, $P =0.020$).

Conclusion These results suggest that PM cross-reacts with the mother’s immune response and impairs her cytokine profile, which might alter maternal haemoglobin levels and the baby's birth weight.

Background

Plasmodium falciparum infections during pregnancy result in pregnancy-associated malaria (PAM) and placental malaria (PM). In PM, infected erythrocytes (IEs) bind to chondroitin sulphate A (CSA) in the placenta [1], which can lead to maternal morbidity and severe fetal and neonatal complications [2]. IEs are sequestered in the placental syncytio-trophoblast with the parasite ligand VAR2CSA [3]. While pregnancy is characterized by an induced immunosuppression [4], an activated immune system during pregnancy might have a role in protection against malaria and poor delivery outcomes [5], and it might decrease the risk of both *P. falciparum* and *Plasmodium vivax* infections [6]. Acquired protection against a range of *P. falciparum* antigens is present during pregnancy in a parity-dependent manner [7]. However, although a significant number of women have antibodies against placental parasites at delivery, their placentas remain infected [8].

It is also known that *P. falciparum* IEs induce inflammation by monocytic infiltration of the malaria infected placenta, which is associated with maternal anaemia and low birth weight (LBW) [9, 10]. This inflammation may influence cellular functions by altering the balance of cytokines and chemokines in the peripheral and

placental blood of the women [11, 12]. Some cytokines have a protective role while others play a pathological role [13-15]. Although the balance between pro- and anti-inflammatory cytokines is a key factor in the regulation of an effective immune response to PM [16], the roles of the produced cytokines are controversial. Among the cytokines that are produced, IL-27 and IL-6 can elicit both pro-inflammatory and anti-inflammatory effects, and high concentrations of IL-6 may protect against PM [17].

IFN- γ and IL-17A levels have been found to be negatively correlated with malaria infection and maternal anaemia [15], and an association of IFN-γ with protection from malaria has been reported [18, 19]. Increased Th1 response due to infection was found to be incompatible with a successful pregnancy in mice [20]. An elevated level of IFN-γ may result in trophoblast damage, preterm delivery, and LBW associated with PM [21-23]. IL-17A may facilitate local inflammation by recruiting and activating immune cells, leading to the upregulation of inflammatory cytokine production [24], but little is known about its role in malaria infection during pregnancy and PM.

Plasmodium falciparum infection during pregnancy increases the placental levels of IL-10 and IL-4 [13, 25]. Elevated levels of the placental plasma anti-inflammatory cytokine, IL-10, were associated with PM, and were implicated in the pathogenesis of severe anaemia [9, 12, 13]. Moreover, IL-10 has been identified as an immunosuppressive cytokine associated with poor pregnancy outcome in a mouse model of PM [26]. Furthermore, placental infection influences fetal immune responses by affecting the Th1/Th2 balance in umbilical cord blood through IL-10 production by T regulatory cells as a result of infection [27].

An understanding of the inflammatory responses in the mother and foetus and the cause of LBW during PM could help optimize efforts to prevent the consequence of placental sequestration that results in poor birth outcomes.

This study aims to investigate the inflammatory environment in maternal peripheral, placental, and umbilical cord blood in response to PM and the extent to which this may influence pregnancy outcomes. The study was conducted with a cohort of Sudanese women from the Blue Nile State, which has a high rate of seasonal malaria transmission.

Methods

Study area and population

This cross-sectional study consisted of a cohort of pregnant women in Blue Nile State, Sudan at their time of delivery, who were recruited between January 2012 and December 2015. The study area, population, and the sample collection method have been described elsewhere [28]. Briefly, a total of 1149 consenting pregnant women were recruited for assessment of the prevalence and risk factors of PM in the study area. The study participants for the sub-study described in this paper included a group of 185 women, who signed informed consent forms and for whom at least 2 ml of peripheral, placental and cord plasma were available, ranging in age between 18 and 22. Information on the mother's socio-demographic characteristics, such as age, parity, education, use of bed nets and anti-malarial drugs, was obtained via a

questionnaire. The haemoglobin level was assessed, and maternal anaemia at delivery was defined as a haemoglobin level <11 g/d. The neonates were weighed immediately after birth.

Sample collection

Maternal peripheral, placental and umbilical cord blood samples were collected immediately after delivery in heparinized vacutainer tubes. A portion of each sample was used to prepare smears for malaria microscopy and to determine the haemoglobin levels in maternal blood.

The rest of the blood was centrifuged at 3,000 g for 3 min, and the undiluted plasma was aliquoted into several tubes (to avoid freeze and thaw) and stored at -80°C until thawed on the day that cytokine assays were performed. The blood smears were routinely stained with Giemsa and microscopically examined by two expert microscopists to determine the presence of malaria parasites.

ELISA malaria antibody test

The Abcam anti-Malaria Human enzyme-linked immunosorbent assay (ELISA) kit, based on binding of the anti-*Plasmodium* antibodies present in the serum or plasma sample to antigens, immobilized on 96-well plates and designed for qualitative measurement of immunoglobulin G (IgG) and immunoglobulin M (IgM) class antibodies, was used. The cut-off control value was calculated by the mean absorbance value of the cut-off control wells. Samples were considered to give a positive signal if the absorbance value was greater than 10% over the cut-off value.

Measurement of the cytokines

The plasma of maternal peripheral, placental and neonate umbilical cord blood was simultaneously subjected to cytokine screening of IL-4, IL-6, IL-10, IL-17A and INF γ using Human ELISA MAX™ Deluxe commercial kits (BioLegend, USA) according to the manufacturer's instructions. The sensitivity of the assay for each cytokine was 2 pg/ml, which was the minimum detectable concentration of each cytokine.

Statistical analysis

Data were analysed using SPSS software for Windows. Continuous data were checked for normality using the Shapiro-Wilk test; they were expressed as median (interquartile) if they were found to not be normally distributed. The Mann-Whitney U test and the Kruskal-Wallis H test (non-parametric) were used to compare the non-normally distributed continuous variables between two and three groups, respectively. Spearman's correlation test was used to assess the correlations between the non-normally distributed continuous variables.

Results

A total of 185 samples were collected from maternal peripheral, placental, and umbilical cord blood. The median (interquartile) of the age, parity, and haemoglobin level of the participants was 20 years (18–22), 2 (1–3), and 10.2 g/dl (9.3–11.3) respectively. A total of 127 (68.6%) women had anaemia (haemoglobin

<11.0 g/dl). Ninety-two (49.7%), 97(52.4%) and 55 (29.7%) of the samples from the maternal peripheral blood, placental, and umbilical cord respectively had blood film positive for *P. falciparum* malaria. Forty-three (23.2%) of the investigated blood films were positive in the three compartments of the same patient.

The median (interquartile) of the birth weight was 2.4 Kg (2.3–2.7). Ninety-eight (53.0%) of the new-borns had LBW (birth weight <2.5 Kg).

With the exception of IL-17A levels, the antibodies and cytokines levels were significantly lower in the umbilical cord samples than the maternal peripheral and placental samples. IL-17A level was similar in the maternal and umbilical cord, however the maternal and umbilical cord level was significantly lower compared with the placental level (Table 1).

While the levels (in three compartments) of antibodies, IL- 4 and IL-10 were significantly higher, the levels of IL-6 in all the compartments and maternal IFN-γ were significantly lower in the malaria positive samples in comparison to the malaria negative samples. Maternal peripheral IL-17A levels and the placental and umbilical IFN-γ levels showed no significant difference between the PM group and the non-infected group (Table 1).

Table 1
Comparing the median (interquartile) antibodies and cytokines level between the maternal, placental and cord compartment and the malaria status

Variables		Maternal	Placental	Umbilical cord	P
Total number		185	185	185	
Number of malaria positive		92	97	55	
Number of Malaria negative		93	88	130	
Antibodies	Total	2.6(1.8 – 3.0)	1.3(1.0 – 1.6)	0.7(0.5 – 0.8)	< 0.001
	Malaria positive	3.1(2.7 – 3.4)	1.4(1.1 – 1.9)	0.8(0.6 – 1.0)	< 0.001
	Malaria negative	1.8(1.4 – 2.4)	1.1(0.9 – 1.5)	0.7(0.5 – 0.8)	< 0.001
	P value	< 0.001	< 0.001	0.005	
IL4	Total	4.2(2.3 – 7.3)	6.7(4.4 – 10.6)	1.9(1.2 – 2.9)	< 0.001
	Malaria positive	6.5(3.9 – 8.5)	10.3(8.3 – 13.3)	2.5(1.8 – 3.6)	< 0.001
	Malaria negative	3.1(1.7 – 4.3)	4.7(3.4 – 6.0)	1.6(1.0 – 2.5)	< 0.001
	P value	< 0.001	< 0.001	< 0.001	
IL 6	Total	39.2(23.7 – 63.4)	19.8(12.3 – 30.6)	8.3(4.1 – 14.2)	< 0.001
	Malaria positive	27.6(20.8 – 40.9)	16.1(9.4 – 20.1)	5.6(2.8 – 11.6)	< 0.001
	Malaria negative	58.3(38.1 – 73.2)	29.4(19.4 – 41.1)	9.5(5.5 – 14.5)	< 0.001
	P value	< 0.001	< 0.001	0.002	
IL10	Total	63.5(31.5 – 102.0)	37.3(17.9 – 71.5)	12.6(7.5 – 25.3)	< 0.001
	Malaria positive	89.4(49.5 – 175.5)	65.4 (36.2 – 102.5)	15.6(9.4 – 37.8)	< 0.001
	Malaria negative	46.5(24.4 – 72.0)	19.4(12.7 – 37.0)	13.5(6.8 – 20.9)	
	P value	< 0.001	< 0.001	0.032	
IL17A	Total	3.2(1.4 – 5.4)	8.6(6.4 – 14.2)	3.3(2.1 – 7.0)	< 0.001

Variables		Maternal	Placental	Umbilical cord	P
	Malaria positive	2.5(1.4 – 5.2)	7.5(5.6 – 8.6)	2.8(2.0 – 3.5)	< 0.001
	Malaria negative	3.6(1.4 – 5.6)	14.3(10.0 – 16.3)	3.6(2.3 – 8.0)	< 0.001
	P value	0.214	< 0.001	0.002	
IFN-γ	Total	68.1(39.1 – 107.0)	86.7(45.9 – 192.7)	27.6(18.4 – 67.8)	< 0.001
	Malaria positive	48.8(32.9 – 78.0)	129.9(36.8 – 281.9)	32.1(17.2 – 96.3)	< 0.001
	Malaria negative	97.2(58.3 – 121.9)	76.1(49.6 – 112.1)	27.5(18.6 – 57.5)	< 0.001
	P value	< 0.001	0.065	0.536	

While the level of maternal antibodies was significantly higher in primipara, there was no significant difference in the placental and umbilical cord antibodies level between primipara and parous women. There was no significant difference in the maternal peripheral and placental IL-17A levels between the primipara women and the parous women. The level of IL-4 was significantly higher in the primipara women in all three types of samples (maternal peripheral, placental, and umbilical cord blood). The IL-6 levels in the maternal peripheral, placental and umbilical cord blood, and the umbilical cord levels of IL-17A were significantly lower in the primipara women. The maternal peripheral and placental IL-10 levels and the placental and umbilical cord IFN-γ levels (no significant difference in the maternal peripheral IFN-γ levels) were significantly higher in the primipara women in comparison to the parous women. (Table 2).

Table 2
Comparing antibodies and cytokines level between primiparas and parous women

Variables		Primiparas (n = 92	Parous (93)	P
Antibodies	Maternal	2.7(2.0 – 3.1)	2.4(1.7 – 3.0)	0.026
	Placental	1.3(1.0 – 1.7	1.3(0.9 – 1.6)	0.066
	Umbilical cord	0.7(0.5 – 0.8)	0.7(0.5 – 0.8)	0.528
IL4	Maternal	4.6(3.2 – 8.1)	3.7(1.8 – 5.7)	0.001
	Placental	7.5 (5.2 – 12.5)	6.2(4.2 – 9.7)	0.014
	Umbilical cord	2.0(1.2 – 3.0)	1.6(1.0 – 2.6)	0.03
IL 6	Maternal	33.2(31.6 – 57.5)	43.9(29.7 – 68.8)	0.007
	Placental	16.5(10.2 – 27.7)	22.3(16.9 – 34.9)	0.005
	Umbilical cord	7.0(3.7 – 12.3)	9.6(4.6 – 15.6)	0.050
IL10	Maternal	70.2(40.5 – 129.5)	51.3(27.6 – 89.7)	0.005
	Placental	45.2(19.7 – 92.0)	32.2(16.4 – 58.1)	0.022
	Umbilical cord	14.3(8.4 – 28.7)	11.0(7.3 – 18.9)	0.077
IL17A	Maternal	3.3(1.3 – 5.9)	2.8(1.4 – 5.1)	0.724
	Placental	8.7(5.9 – 14.3)	8.6(6.4 – 14.0)	0.743
	Umbilical cord	3.1(1.5 – 6.9)	3.6(2.5 – 7.1)	0.027
IFN-γ	Maternal	63.7(34.3 – 98.3)	73.3(44.5 – 111.5)	0.231
	Placental	180.9(58.4 – 292.7)	59.3(37.7 – 87.8)	< 0.001
	Umbilical cord	37.3(19.7 – 96.3)	23.7(19.5 – 48.7)	0.005

The antibody levels in the plasma of the maternal peripheral, placental and umbilical cord blood samples were significantly positively correlated with the IL-4 levels as well as with the maternal and placental levels of IL-10. There was no correlation between the umbilical cord antibody levels and the umbilical cord level of IL-10 or the IL-17A levels in the maternal peripheral, placental and umbilical cord blood samples. The maternal and placental antibody levels were significantly negatively correlated with maternal and placental levels of IL-6. The maternal peripheral of the antibodies levels was significantly negatively correlated with the maternal level of IFN-γ. There was no significant correlation between the umbilical cord level of antibodies and the umbilical cord level of IL 6. There was no correlation between the placental and umbilical cord level of antibodies and placental and umbilical cord level IFN-γ (Table 3, Table 4, Table 5).

Table 3
Correlations of the maternal plasma levels of antibodies, cytokines, maternal hemoglobin and birth weight

Variables	Hemoglobin	Birth weight	Antibodies	IL4	IL 6	IL10	IL17A	IFN-γ
	r	*r*		*r*	*r*	*r*	*r*	*r*
	P	*P*		*P*	*P*	*P*	*P*	*P*
Hemoglobin		0.749	− 0.518	− 0.544	0.430	− 0.478	0.130	0.171
		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.078	0.020
Birth weight	0.749		− 0.584	− 0.525	0.455	− 0.484	0.129	− 0.233
	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	0.080	0.001
Antibodies	− 0.518	− 0.584		0.438	− 0.342	0.361	− 0.072	− 0.228
	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	0.329	0.002
IL4	− 0.544	− 0.525	0.438		− 0.476	0.438	0.034	− 0.142
	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	0.647	0.054
IL 6	0.430	0.455	− 0.342	− 0.476		−0.449	0.151	0.204
	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	0.040	0.005
IL10	− 0.478	− 0.484	0.361	0.438	− 0.449		− 0.110	− 0.056
	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.135	0.448
IL17A	0.130	0.129	− 0.072	0.034	0.151	− 0.110		0.063
	0.078	0.080	0.329	0.647	0.040	0.135		0.395
IFN-γ	0.171	-0.233	− 0.228	− 0.142	0.204	− 0.056	0.063	
	0.020	0.001	0.002	0.054	0.005	0.448	0.395	

Table 4
Correlations of the placental plasma levels of antibodies, cytokines, maternal hemoglobin and birth weight

Variables	Hemoglobin	Birth weight	Antibodies	IL4	IL 6	IL10	IL17A	IFN-γ
	r	*r*		*r*	*r*	*r*	*r*	*r*
	P	*P*		*P*	*P*	*P*	*P*	*P*
Hemoglobin	1.000	0.749	− 0.351	− 0.586	0.403	− 0.436	0.377	− 0.146
	.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.047
Birth weight	0.749	1.000	− 0.417	− 0.574	0.402	− 0.445	0.391	-0.146
	< 0.001	.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.048
Antibodies	− 0.351	− 0.417	1.000	0.307	− 0.165	0.146	− 0.097	− 0.003
	< 0.001	< 0.001	.	< 0.001	0.025	0.047	0.189	0.966
IL4	− 0.586	− 0.574	0.307	1.000	− 0.406	0.462	− 0.357	0.185
	< 0.001	< 0.001	< 0.001	.	< 0.001	< 0.001	< 0.001	0.012
IL 6	0.403	0.402	−0.165	− 0.406	1.000	− 0.393	0.335	− 0.199
	< 0.001	< 0.001	0.025	< 0.001	.	< 0.001	< 0.001	0.007
IL10	− 0.436	− 0.445	0.146	0.462	− 0.393	1.000	− 0.446	0.299
	< 0.001	< 0.001	0.047	< 0.001	< 0.001	.	< 0.001	< 0.001
IL17A	0.377	0.391	− 0.097	− 0.357	0.335	− 0.446	1.000	− 0.117
	< 0.001	< 0.001	0.189	< 0.001	< 0.001	< 0.001	.	0.114
IFN-γ	− 0.146	-0.146	− 0.003	0.185	− 0.199	0.299	− 0.117	1.000
	0.047	0.048	0.966	0.012	0.007	< 0.001	0.114	.

Table 5
Correlations of the umbilical cord plasma levels of antibodies, cytokines, maternal hemoglobin and birth weight

Variables	Hemoglobin	Birth weight	Antibodies	IL4	IL 6	IL10	IL17A	IFN-γ
	r	*r*		*r*	*r*	*r*	*r*	*r*
	P	*P*		*P*	*P*	*P*	*P*	*P*
Hemoglobin	1.000	0.749	−0.248	−0.512	0.334	−.0300	0.460	−0.073
	.	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.322
Birth weight	0.749	1.000	−0.304	−0.493	0.370	−0.329	0.500	-0.069
	< 0.001	.	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.035
Antibodies	−0.248	−0.304	1.000	0.196	−0.106	−0.010	−0.053	0.036
	< 0.001	< 0.001	.	0.007	0.152	0.889	0.472	0.623
IL4	−0.512	−0.493	0.196	1.000	−0.316	0.294	−0.304	0.140
	< 0.001	< 0.001	0.007	.	< 0.001	< 0.001	< 0.001	0.058
IL 6	0.334	0.370	−0.106	−0.316	1.000	−0.248	0.333	−0.024
	< 0.001	< 0.001	0.152	< 0.001	.	0.001	< 0.001	0.741
IL10	−0.300	−0.329	−0.010	0.294	−0.248	1.000	−0.324	0.215
	< 0.001	< 0.001	0.889	< 0.001	0.001	.	< 0.001	0.003
IL17A	0.460	0.500	−0.053	−0.304	0.333	−0.324	1.000	−0.136
	< 0.001	< 0.001	0.472	< 0.001	< 0.001	< 0.001	.	0.065
IFN-γ	−0.073	-0.069	0.036	0.140	−0.024	0.215	−0.136	1.000
	0.322	0.035	0.623	0.058	0.741	0.003	0.065	.

The IL-4 levels in the plasma of the three types of blood samples were significantly negatively correlated with the IL-6 levels, and they were positively correlated with the IL-10 levels. There was a borderline negative correlation between the maternal peripheral IL-4 levels and the maternal peripheral IFN-γ levels. A negative correlation was found between the placental IL-4 levels and the placental IL-17A levels. A positive

correlation was found between placental IL-4 levels and the placental IFN-γ level. In the umbilical cord samples, the level of IL-4 was negatively correlated with the level of IL-17A.

The IL-6 levels in all three types of the blood samples were significantly negatively correlated with the levels of IL-10 and positively correlated with the levels of IL-17A. The maternal and placenta IL-6 levels were positively and negatively, respectively correlated with maternal and placenta IFN-γ.

While no correlation was found between the maternal peripheral level of IL-10 and the maternal peripheral levels of IL-17A and IFN-γ, negative correlations were found between the placental and umbilical cord levels of IL-10 and IL-17A, and a positive correlation was found between IL-10 levels and the level of placental and umbilical cord of IFN-γ. No correlation was found between the level of IL-17A for the maternal peripheral, placental and umbilical cord blood samples and the IFN-γ levels of the plasma in the three types of samples.

Maternal haemoglobin was significantly negatively correlated with the levels of antibodies, IL-4 and IL-10 in the maternal peripheral, placental and umbilical cord blood samples; however, no significant correlation was found between maternal haemoglobin and the level of the peripheral IL-17A. Significantly positive and negative correlations were found between the levels of IL-6 and IFN-γ, respectively, and birth weight for all three types of samples, which was significantly negatively correlated with the levels of antibodies, IL-4 and IL-10. While no significant correlation was found between birth weight and maternal levels of IL-17A, it was significantly positively correlated with the levels of IL-17A in the placental and umbilical cord samples (Table 3, Table 4, Table 5).

Discussion

The present study found that malaria infection altered the investigated cytokines (IL-4, IL-6, IL-10, IL-17A and INF-γ), with elevated levels of antibodies to recombinant antigens in Sudanese maternal, placental and neonatal plasma. The high levels of the pregnancy non-specific antibodies detected in primipara peripheral compared to parous can be explained by the high malaria transmission in the study area. The presence of non-specific, non-protective Ig may delay or interfere with the acquisition of memory B cells [29].

In the current study, the levels of IL-4 and IL-10 were elevated but the levels of IL-6 and IL-17 were reduced in the plasma of the maternal peripheral, placental and umbilical cord blood samples of the PM infected mothers in comparison to the non-infected group. Moreover, the maternal peripheral level of IL-17A and the placental and neonate IFN-γ levels were not significantly different due to the infection. Several previous studies have shown elevated levels of type 2 anti-inflammatory cytokines IL-10 and IL-4 in the malaria-infected group [12, 13, 15, 23, 25, 30, 31]. Conversely, the present results were different from the previous findings in an area with unusable malaria transmission in Sudan, where higher levels of IL-4 and IL-10 in the non-infected group was reported [11]. The findings of the increased IL-6 levels in non-infected women is in agreement with the results of other studies [11, 16, 32, 33].

Interestingly, the level of cytokines in the maternal peripheral samples among the investigated groups was significantly correlated with the levels in the placental and umbilical cord samples. Nevertheless, plasma from the umbilical cord (except for the maternal peripheral IL-17A levels) contained significantly lower concentrations of the investigated cytokines in comparison to the peripheral and placental plasma in both study groups (PM infected and non-infected groups). Kabyemela *et al*. [25], in Tanzania, and Ibitokou [32], in Benin, reported similar levels of IFN-γ between the investigated groups. The results of the present study corroborate the findings of a previous study that reported higher levels of IFN-γ in infected placentas [9], and a study from Cameron that found an increase in maternal IFN-γ and IL-17-A levels in non-infected controls [15]. The elevated detection levels of IFN-γ in the investigated samples may be due to the ability of the innate immune response to produce IFN-γ to clear the parasite, probably as the first line of defense against both peripheral and placental infection [18]. It has been suggested that the differences in cytokine responses associated with malaria infection have a gravidity-based pattern [22].

Infiltration of immune cells in the placental intervillous spaces, due to the sequestration of *P falciparum* IEs, disrupting Th1 and Th2 cytokines balance in both placental and peripheral blood [22, 34]. Contradictory findings about impairment of cellular immune responses against malaria in pregnancy and PM have been described [12, 13, 15, 17, 21, 25, 35, 36]. However, the differences in the cytokine profiles between these studies may be attributed to variations in the cytokine measurement methods used and the malaria endemicity in different study areas, as well as differences in the diagnostic techniques and the study population.

In the present study, among the cytokines assessed, IL-4 and IL-10 were elevated and significantly correlated ($P < 0.001$) in the plasma of the three types of blood samples that were investigated; no correlation was found between the maternal peripheral levels of IL-10 and IFN-γ ($P=0.448$). IL-17A was positively correlated with IL-6, but no correlation was found between the IFN-γ in the plasma of the three types of samples. IFN-γ and IL-17-A are both effector helper T cells, and IL-17A can facilitate regulation of inflammatory cytokine production by accelerating specific inflammation via the recruitment and activation of immune cells. IL-6 is both a target of IL-17 and a differentiation factor for Th17 cells that led to an increase in IL-17 [37]. Agudelo *et al*. [23] observed a significant correlation between IL-4 and IL-10 in the placental samples, but not in the peripheral blood, and levels of maternal IL-10 and IFN-γ were positively correlated. Moreover, [23] reported high expression of IFN-γ, TNF and IL-10 in the placental tissues and peripheral blood samples, and placental IL-4 in the infected women in comparison to the non-infected group. Due to the high expression associated with PM infection, it has been suggested that the IL-10 level in peripheral blood to be used as biomarker of placental inflammation related to PM [25] or as an immunosuppressive factor [26].

The antibody levels were negatively correlated with the IL-6 and IFN-γ levels in the present study. Chandragiri *et al.* [30] also reported a negative correlation between the same cytokines and non-pregnancy-specific variant surface antigens (VSA) in women with severe malaria in areas characterized by unstable malaria transmission in Sudan.

The present study found that IL-6 and IFN-γ had significant effects on birth weight and the maternal haemoglobin level, accompanied by the negative correlation of IL-4 and IL-10 in the plasma of the three

types of samples investigated. It has been documented that there is a disturbance of cytokine equilibrium in malaria during pregnancy, and PM may be involved in many pathological disorders; it may also have negative consequences, such as LBW and reduced maternal haemoglobin level [38-40].

While the levels of IL-17A in the placental and umbilical cord samples were significantly positively correlated with birth weight and maternal haemoglobin, no significant correlation was found with the IL-17A levels in the maternal peripheral samples. The roles of IFN-γ and IL-10 in the malaria-infected women with maternal anaemia and baby birth weight was controversially documented in a reviewed by Seitz *et al.* [40]. Although Djontu *et al.* [16] reported no significant association between IL-6 level, maternal haemoglobin and baby birth weight, an elevated level of IL-6 was associated with anaemia in another study [30].

Similar to the current study's findings, an association was reported between maternal haemoglobin and IL-17A levels and the peripheral plasma level of IFN-γ. It has been suggested that both cytokines provide protection against infection [15]. Furthermore, elevated levels of IL-17 with high levels of IL-4, IL-12 and IFN-γ were associated with haemoglobin loss in malaria recovered semi-immune mice [41]. Fitri *et al.* [42] reported that an imbalance between IL-17 and IL- 10 caused low fetal weight in *Plasmodium berghei* infection in mice.

Disturbance of proinflammatory cytokines and the inflammatory disorder of iron haemostasis led to the development of malarial anaemia [43]. Elevated levels of circulating IL-6, which play a vital role in T cells differentiation and immune response polarization, have been strongly related to reduced haemoglobin concentration in reticulocytes.

There are limitations to this study to be addressed. The study did not investigate the antibodies related to the pregnancy-specific antigens, particularly VAR2CSA, to determine their correlation with the cytokine response associated with infection. The study was conducted in a setting of other common infections during pregnancy which could influence variations in antibodies and / or cytokine levels, not checking for the presence of other infections is another limitation.

Conclusion

In the present study, maternal peripheral infection and PM cross-react with the mother's immune response to stimulate an immune activation response accompanied by secretion of various cytokines in the maternal peripheral, placental and umbilical cord blood in Sudanese women. The present findings support the evidence reported in previous studies, which found that PM affects cytokines levels in infected women. However, longitudinal studies are needed to understand the maternal immune response throughout the entire course of pregnancy.

Declarations

Ethical Approval

The study received ethical approval from the Ethical and Scientific Committees of the Tropical Medicine Research Institute, National Centre for Research, and the Directorate of Research, Federal Ministry of Health, Khartoum, Sudan. Written informed consent was obtained from all participants before inclusion in the study.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interests

The authors declare that they have no conflict interests.

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Authors' contributions

OS, CF, MI, and AI conceived the study and participated in the study coordination. AM conducted the clinical work. OM participated in the immunological laboratory work. OS, IA and NA analyzed and interpreted the data. NA shared the co-drafting of the manuscript. All authors revised and approved the final draft of the manuscript.

23.2%) of the investigated blood films were positive in the three compartments of the same patient.

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