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Associations between maternal inflammation during pregnancy and infant birth outcomes in the Seychelles Child Development Study'

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Abstract

Problem—Markers of maternal inflammation may determine infant birth outcomes.

Method of Study—Maternal serum samples were collected at 28 weeks gestation ($n=1418$) in the Seychelles Child Development Study Nutrition Cohort 2 and analyzed for immune markers by MSD multiplex assay, including cytokines from the Th1 (IFN- γ , IL-1 β , IL-2 and TNF- α) and Th2 (IL-4, IL-5, IL-10) subsets, with IL-6, MCP-1, TARC, sFlt-1 and VEGF-D. Associations of log-transformed immune markers with birthweight, length, head circumference and gestational age were assessed by multiple linear regression models, which were adjusted for maternal age, BMI, parity, child sex, gestational age and socioeconomic status.

Results—Neither total Th1, Th2 nor Th1:Th2 were significantly associated with any birth outcome. However, the angiogenesis marker VEGF-D was predictive of a lower birthweight ($\beta=-0.058$, $P=0.017$) and birth length ($\beta=-0.088$, $P=0.001$) after adjusting for covariates. Higher concentrations of CRP were predictive of a lower birthweight ($\beta=-0.057$, $P=0.023$) and IL-2 ($\beta=0.073$, $P=0.009$) and the chemokine MCP-1 ($\beta=0.067$, $P=0.016$) were predictive of a longer gestational age.

Conclusions—In our cohort of healthy pregnant women, we found no evidence for associations between the Th1 or Th2 inflammatory markers with birth outcomes. However, VEGF-D and CRP

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

appear to predict lower birthweight and IL-2 and MCP-1 a longer gestation. Greater understanding is required of the variation in these immune markers at different gestational stages, as well as the factors which may regulate their balance in healthy pregnancy.

n=233

Keywords

Inflammation; Pregnancy; Birth Outcomes; Cytokines; Seychelles Child Development Study

Introduction

During healthy pregnancy, the immune system adapts to protect both the mother and fetus. The CD4⁺ T cells, involved in the T helper type 1 (Th1) mediated response, secrete inflammatory cytokines, including the pro-inflammatory IFN- γ and TNF- α , which promote host defence against intracellular viral and bacterial pathogens. Cytokines (IL-4, IL-5 and IL-10) derived from the T helper type 2 cells (Th2) tend to be less pro-inflammatory and are important for antibody-mediated defence against large extracellular pathogens and allergic responses (Sykes *et al*, 2012). The Th1:Th2 ratio is often used to characterize dominating cytokine patterns. The first trimester of pregnancy is believed to be predominantly a Th1 phase as implantation occurs triggering the innate immune system to stimulate monocyte release and a number of other cells (Saito *et al*, 1999; Mor and Cardenas, 2010; Sacks *et al* 1999). This response is followed by an anti-inflammatory immunological phase where there is bias towards Th2 cytokine production and down-regulation of Th1 cytokines (Mor and Cardenas, 2010). The immune response returns to a Th1 phase in late pregnancy, promoting contraction of the uterus and initiation of labour (Mor *et al*, 2011).

Evidence suggests that the maternal inflammatory cytokine profile and resulting Th1:Th2 balance is important for successful pregnancy and birth outcomes (Cappelletti *et al*, 2016). Studies have reported higher third trimester concentrations of Th1 cytokines in cases of miscarriage and pre-eclampsia than in healthy term pregnancies (Halonen *et al*, 2009). Similarly, greater maternal concentrations of Th1 cytokines in early pregnancy have been associated with preterm birth and lower birthweight and length (Wilkinson *et al*, 2017). Any factor with the potential to shift the Th1:Th2 balance in favour of Th1, such as hormonal changes, pathogens or environmental stimuli, may increase susceptibility to inflammatory conditions and adversely affect pregnancy outcomes (Challis *et al* 2009; Robinson & Klein, 2012). Other immune cells are active at the maternal-fetal interface, including chemokines and angiogenesis markers, and together promote fetal tolerance and the maintenance of normal pregnancy (Du *et al*, 2014). Understanding more on how the maternal immune response might influence fetal growth might help prevent adverse fetal growth outcomes. There remains very limited data from human studies on concentrations of immune markers in healthy pregnancy and even less on their potential impact on fetal growth. In the present study, we characterized an extensive panel of immune markers in 28 weeks gestational blood samples with the aim of investigating relationships between maternal inflammation and birth outcomes including weight, length, head circumference and gestational age.

Materials and Methods

Study Population

Nutrition Cohort 2 (NC2) is part of the Seychelles Child Development Study, a multi-cohort observational study with the overall aim of investigating associations between prenatal methylmercury (MeHg) exposure and child neurodevelopment. NC2 was conducted on Mahé, the main island of the Republic of Seychelles, where mothers were recruited during their first antenatal visit (from 14 weeks gestation) at eight health centres between 2008 and 2011. Inclusion criteria for NC2 included being native Seychellois, being ≥ 16 y of age, having a singleton pregnancy, and with no obvious health concerns (Strain *et al.*, 2015). The study was reviewed and approved by the Seychelles Ethics Board and the Research Subjects Review Board at the University of Rochester.

Inflammatory Markers

Non-fasting blood samples were drawn from mothers at approximately 28 weeks gestation and analyzed for inflammatory markers as previously described (Irwin *et al.*, 2019). Cytokines that were measured for cell-mediated inflammatory responses (Th1) include interleukin (IL)-1 β , interleukin (IL)-2, interferon-gamma (IFN γ), and tumour necrosis factor alpha (TNF- α). The cytokines associated with humoral response (Th2) included IL-4, IL-5 and IL-10. In addition, we measured IL-6, which plays a dual role in both Th1 and Th2 cell differentiation (Dielh & Rincon, 2002). We measured two pregnancy-associated chemokines, monocyte chemotactic protein-1 (MCP-1) and thymus activated regulated chemokine (TARC) and two angiogenesis markers noted to be important for maintenance of placental vascular development and blood flow, soluble fms-like tyrosine kinase-1 (sFlt-1) and a member of the vascular endothelial growth factor (VEGF) family, VEGF-D. All markers were analyzed as continuous variables in pg/mL, with CRP measured in mg/L.

Birth Outcomes

Birthweight (g), length (cm) and head circumference (cm) were assessed at birth by trained nurses to the nearest two decimal places using routine clinical procedures and standardized scales. For descriptive purposes, we calculated sex-specific percentiles of birthweight for gestational age by using US national reference data (Oken *et al.*, 2003). We defined small-for-gestational age (SGA) as birthweight for gestational age and sex below the 10th percentile and large-for-gestational age (LGA) as above the 90th percentile. Those within the 10th and 90th percentiles were classed as appropriate-for-gestational age (AGA).

Covariates

Mothers reported information about their age, parity, smoking status and alcohol use at enrolment to the study using questionnaires administered by trained nurses. Child sex was recorded at birth along with birth outcome data. When their infant was approximately 20 months of age Hollingshead socioeconomic status (SES) was assessed using a modified index relevant to the Republic of Seychelles (Davidson *et al.*, 1998). We combined occupational and educational codes as previously described into a continuous SES score (Davidson *et al.*, 1998). Height and weight of the mothers were obtained from which their

postnatal BMI was calculated ($\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$). We adjusted for BMI, as measured at 20 months postnatally, based on data from our previous study showing a high correlation between BMI assessed in early pregnancy and postnatal BMI (Davidson *et al.*, 2008). Gestational age (weeks) was recorded at delivery.

Statistical Analysis

From our final database ($n=1536$), we excluded mother-child pairs where mothers were <16 years of age ($n=1$), did not give consent ($n=1$), had gestational problems ($n=3$), where the infant was deceased at birth ($n=5$), was classed as a preterm birth (born <34 weeks; $n=19$), a twin birth ($n=34$), or had a birthweight $<1500\text{g}$ ($n=2$) and those missing all inflammatory and/or birth outcome data ($n=49$). Two mothers were removed with extremely high concentrations of all inflammatory markers indicating possible illness. Two mothers were removed with questionable dates of gestational week at blood collection and seven infants with questionable head circumference measurements at birth were removed ($>38\text{cm}$), leaving $n=1411$ mother-child pairs for analysis. For our analysis, we summed cytokines to determine the Th1:Th2 ratio as follows: Th1-type cells: $\text{IL-1}\beta + \text{IL-2} + \text{IFN-}\gamma + \text{TNF-}\alpha$ and Th2-cells: $\text{IL-4} + \text{IL-5} + \text{IL-10}$. We analyzed the remaining markers individually: CRP, IL-6, MCP-1, TARC, sFlt-1 and VEGF-D. Distributions of maternal and child characteristics were first examined, followed by Pearson's correlations to examine bivariate associations of each covariate with birth outcomes. One-way ANOVA was used to compare concentrations of immune markers between mothers whose infants were classified as either SGA, AGA or LGA. We conducted multiple linear regression models by evaluating each maternal inflammatory marker as continuous data, both individually as well as the Th1 and Th2 sums and the Th1:Th2 ratio. For each marker, we ran main effects models both with and without adjustment for the covariates known to be associated with birth outcomes (child sex (M/F), maternal age at enrolment, gestational age, parity, SES and maternal BMI). We also examined gestational age as a birth outcome in itself and in this model, we adjusted for child sex, maternal age, parity, SES and maternal BMI. We added a constant of +1 to all inflammatory marker data, including Th1, Th2 and Th1:Th2, prior to log-transformation. Undetectable immune marker data are common in multiplex assays; however the Meso Scale Discovery (MSD) Platform is extremely sensitive and as such, 80% of concentrations were above the LLOD for most markers. For undetectable values we inputted $\text{LLOD} / 2$ as described by others (Ogden, 2010). The lowest rates of detection were observed for IL-2 and IL-4 where only 36% of all samples were above the LLOD. Excluding these markers from Th1, Th2 and Th1:Th2 variables did little to impact the relationships with birth outcomes. Therefore, we included these markers both as individual variables and within the Th1 and Th2 variables.

Results

Table 1 shows the descriptive characteristics of mothers and infants. Among the 1411 mothers with available data, there were $n=734$ male and $n=677$ female children. The mean $\pm$ SD age of the mothers at enrolment to the study was 27 ± 6 years, with a gestational age of 39 ± 1 weeks. Calculating sex-specific percentiles of birthweight for gestational age classified the majority ($n=1101$, 78%) of infants in the cohort as AGA (10–90th percentile)

based on US reference data (Oken *et al*, 2003). When we compared immune markers between mothers according to percentile of birthweight for gestational age, there were no significant differences in maternal concentrations of any inflammatory marker among these categories. Table 2 reports the unlogged immune marker concentrations for the 1411 mothers with complete data showing the assay performance data. The median (25th, 75th percentiles) Th1 and Th2 sums were 9.67 (5.75, 14.75) and 2.14 (1.04, 3.31) pg/mL, with a median Th1:Th2 ratio of 5.16 (3.04, 8.48). Pearson correlation analysis showed maternal age, BMI, parity, child sex (being male) and gestational age to each be significantly correlated with a greater birthweight, length and head circumference ($P<0.01$) (Table 3).

Table 4 presents the results of linear regression models for log-transformed inflammatory marker data with adjustment for covariates. Cytokines grouped as Th1, Th2 or Th1:Th2 did not significantly predict any birth outcome. Increasing concentrations of maternal VEGF-D ($\beta=-0.058$, $P=0.017$) and CRP ($\beta=-0.059$, $P=0.02$) were significantly associated with a lower birthweight, when adjusted for covariates. Maternal VEGF-D was also predictive of a shorter birth length ($\beta=-0.089$, $P=0.001$). Increasing concentrations of maternal IL-2 and MCP-1 were predictive of a longer gestational age (adjusted model $\beta=0.073$, $P=0.009$ and $\beta=0.069$, $P=0.013$). In the unadjusted model, IL-10 was significantly associated with a shorter birth length ($\beta=-0.054$, $P=0.044$) and gestational age ($\beta=-0.054$, $P=0.044$) (data not shown). There was a trend for the association between IL-10 and gestational age to remain following adjustment for covariates ($\beta=-0.049$, $P=0.079$).

Discussion

In our cohort of healthy Seychellois mothers, we found no consistent evidence that maternal markers of inflammation, grouped as the Th1, Th2 or the Th1:Th2 ratio, at 28 weeks gestation were associated with infant birth outcomes. We found that maternal CRP, an acute-phase inflammatory protein indicating an immune response to infection or antigen, was associated with a lower birthweight after adjusting for common confounders. This finding is in agreement with other studies that have measured CRP in each trimester and found it to be associated with a lower birthweight (Ernst *et al* 2011; Ali *et al* 2015; Retnakaran *et al* 2012; de Oliveira *et al*, 2017), and preterm birth (Amarilyo *et al*, 2010). One possible mechanism for the role of prenatal inflammation in determining infant birth outcomes is that infiltration of inflammatory cells to the maternal-fetal tissues can generate vascular dysfunction, which compromises placental blood supply and in turn, affects fetal growth (de Oliveira *et al*, 2017). Birthweight is a known predictor of child morbidity and mortality and being born SGA increases the risk of neonatal complications and lower cognitive development (McCormick *et al*, 1996; Pryor *et al*, 1995). Although CRP is recognized to increase gradually throughout pregnancy in approaching labor, it is suggested that prolonged excessive concentrations at any stage of gestation may be associated with adverse birth outcomes (de Oliveira *et al*, 2015). However, serum CRP is a nonspecific marker of inflammation in the body, which does not identify the stimulus for its elevation and therefore cannot inform as to the site of inflammation (Hackney *et al*, 2008). It is possible that elevated CRP in the mother at 28 weeks gestation may not directly restrict fetal growth, but its elevation may be in response to unidentified damaged tissue, nuclear antigens or

pathogenic organisms. In our study all mothers' CRP concentrations were <10mg/L, which is considered normal and not indicative of acute inflammation (WHO, 2014).

In conjunction with this finding, we found that increased maternal concentrations of the angiogenic growth factor VEGF-D were associated with a lower birthweight and birth length, with a trend for a lower head circumference. The VEGF family plays a role in the early development of the placental-fetal circulatory system, while the VEGF-D isoform is predominantly a lymphangiogenic growth factor (Shibuya, 2013). A splice variant of the VEGF receptor 1, fms-like tyrosine-kinase receptor (FLT-1), soluble FLT-1 (sFlt-1) has anti-angiogenic properties. An imbalance in maternal angiogenic factors, with excess sFlt-1 and decreased VEGF, has been associated with restricted fetal growth and together, these markers may play a pathological role in pre-eclampsia (Wang *et al*, 2009; Shange *et al*, 2017). A number of studies have reported, contrary to our finding, that maternal serum VEGF concentrations are positively correlated with birthweight (Wheeler *et al*, 1999; Rosso *et al*, 1993), including one study which found cord VEGF to be positively correlated, and sFlt-1 negatively correlated with birthweight (Voller *et al*, 2014). However, the literature remains controversial with regards the associations of individual VEGF isoforms at different stages of pregnancy with birth outcomes (Andraweera *et al*, 2012). The location of VEGF expression varies throughout gestation with VEGF-D mainly expressed in the trophoblasts, decidual stromal cells, endothelial cells and vascular smooth muscle cells (Schiessi *et al*, 2009). The majority of evidence suggests that VEGF-D is upregulated in early embryonic development, but less is known about concentrations in later pregnancy. However, angiogenesis is believed to switch from branching to non-branching from 25 weeks gestation onwards and this is accompanied by an increase in sFlt-1 and a decrease of VEGF-A (Andraweera *et al*, 2012). Given that sFlt-1 is an agonist of VEGF, these findings might suggest that VEGF-D is downregulated in later pregnancy and subsequently, that elevated concentrations might be undesirable. Measuring the other family of VEGF markers, VEGF-A, and VEGF-C, might be necessary to understand our finding.

We found MCP-1 and IL-2 to be associated with a longer gestational age. IL-2 is essential for activation and function of the regulatory T cells (Treg) which play a key role in sustaining fetal tolerance and preventing abnormal immune response in pregnancy (Alijotas-Reig *et al*, 2014; Chinen *et al*, 2016). MCP-1 is a chemokine which regulates trophoblast activation and growth of the placenta. Downregulation of MCP-1 is implicated in abnormal fetal growth, with lower maternal and fetal concentrations found in cases of intra-uterine growth restriction (IUGR) (Briana *et al*, 2007). The association of both of these markers with a longer gestational duration is positive and may suggest that these cytokines have actions that could prevent early parturition, perhaps through regulatory effects on the chemotaxis of other immune cells.

The immune response in pregnancy is a state of careful balance between immune cells to reduce the risk of fetal rejection and increase the transfer of maternal antibodies to the fetus (Robinson & Klein, 2012; Mor *et al*, 2011). It is generally believed that the balance of Th1 to Th2 cytokines is in preference of the more pro-inflammatory Th1 cells in early gestation, shifting in the second trimester to a more Th2-dominant response, until labor when the balance shifts in favour of Th1 once more (Chen *et al*, 2012). Elevated Th1 cells in

pregnancy have been associated with pre-eclampsia and adverse birth outcomes (Saito *et al*, 1999; Cappelletti *et al*, 2016). In this study, we found no evidence that maternal Th1:Th2 ratio is associated with infant birth outcomes. It is important to recognize that our cohort differs to other populations in relation to their high fish consumption and intake of long chain n-3 PUFA, which could affect the inflammatory state as previously discussed (Strain *et al*, 2015). Adding further to the complexity of immune adaptations taking place in pregnancy is the balance between chemokines and angiogenesis factors, which are not well understood. Our results showing VEGF-D to be associated with a lower birthweight and length may be explained by endothelial dysfunction, rather than inflammation. It is also important to recognize that the Th1:Th2 paradigm has been challenged in recent years, with knowledge that additional regulatory mechanisms involving Treg cells are just as important for successful pregnancy (Guerin *et al*, 2009), and thus adding a further element of complexity to this area.

A strength of our research is that we examined a broad spectrum of immune markers by sensitive assay approach. Th1:Th2 ratios found in our study at 28 weeks were similar to ratios reported for healthy pregnant women in the third trimester by Saito *et al* (1999), who used flow cytometry to quantify Th1 and Th2 cells. However, our data is limited to one time point in pregnancy and we lack information on the participants' general health and possible presence of infection at the time of blood collection, which could have aided interpretation of our results. De Oliveira *et al* (2017) have suggested that inflammatory markers are simply mediators of the duration of pregnancy and may be predictive of preterm labor. However, in our cohort we excluded preterm births and adjusted for gestational age in our birthweight model. The majority of infants were classed as being an appropriate birthweight for gestational age (AGA) and to the best of our knowledge, mothers were healthy with only 6 cases of gestational diabetes in our cohort and with no known cases of preeclampsia.

Finally, it is possible that the cytokines measured in maternal serum do not closely reflect what is occurring at the maternal-fetal interface (Lin *et al*, 1993). Therefore, future studies should consider measurement of these markers in neonatal blood to help enhance understanding on the mechanisms of these associations with birth outcomes.

Conclusion

Maternal inflammation is a known risk factor for poorer birth outcomes and preterm birth, yet the mechanisms underlying these relationships are complex. Our results from the SCDS NC2 cohort of healthy mothers and children suggest that there are no associations between Th1 and Th2 cytokines at 28 weeks gestation, and birth outcomes. However, CRP and VEGF-D appear to be independent predictors of lower birthweight and length, and MCP-1 and IL-2, of a longer gestational age. The mechanisms underlying these associations warrant further investigation, including studies of these markers at different gestational stages and in cord blood.

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Highlights

- Maternal inflammatory markers, as characterized by the Th1, Th2 and Th1:Th2 ratio at 28 weeks gestation, were not associated with infant birthweight, birth length or head circumference.
- Higher concentrations of the pro- lymphangiogenic vascular endothelial growth factor D (VEGF-D) appear to be predictive of a lower birthweight and length.
- Similarly, greater concentrations of C-Reactive Protein (CRP), which is a pro-inflammatory marker, were associated with a lower birthweight.
- A longer gestational age were predicted by higher concentrations of pro-inflammatory chemokine monocyte chemotactic protein-1 (MCP-1) and interleukin 2 (IL-2).
- Further research is required to aid understanding of the importance of these findings.

Table 1.

Descriptive characteristics of the NC2 mother-child cohort

	<i>n</i>	Frequency <i>n</i> (%)	Mean	SD	Median	25th	75th
Maternal age (yrs)	1410		26.88	6.28	25.92	21.96	31.35
Height (cm)	1312		161.91	6.07	162.00	158.00	166.00
Weight (kg)	1301		70.64	17.90	67.90	57.15	81.35
BMI (kg/m ²)	1301		26.93	6.54	26.15	21.82	30.93
Smoker during pregnancy	1411	12 (0.9)					
Alcohol use during pregnancy	1411	60 (4.3)					
Gestational diabetes	1411	6 (0.4)					
Hollingshead SES	1364		32.01	10.38	31.50	24.00	39.50
Parity	1396		1.00	1.00	1.00	0.00	1.00
Gestational age (wks)	1399		39.06	1.39	39.00	38.00	40.00
Gestational age at blood collection	1379		27.90	1.14	27.86	27.43	28.43
Head circumference (cm)	1385		33.76	1.52	34.00	33.00	35.00
Birth length (cm)	1385		51.12	3.19	51.00	49.00	53.00
Birth weight (kg)	1409		3.19	0.46	3.20	2.89	3.49
Small for Gestational Age (SGA)	1396	249 (17.6)					
Appropriate for Gestational Age (AGA)	1396	1101 (78)					
Large for Gestational Age (LGA)	1396	46 (3.3)					
Female infant	1411	677 (48)					

Table 2.

Maternal serum inflammatory concentrations (pg/ml)

	<i>n</i>	Assay LLOD	Detectable above LLOD (% total assayed)	Mean	SD	Median	25th	75th
IFN- γ	1411	0.20	84.4	5.54	18.98	2.77	0.99	4.93
IL-1 β	1411	0.04	82.6	0.32	0.62	0.19	0.03	0.39
IL-2	1411	0.09	36.1	0.28	0.58	0.06	0.06	0.23
IL-4	1411	0.02	35.5	0.14	0.54	0.01	0.01	0.05
IL-6	1411	0.06	88.7	0.99	1.61	0.52	0.22	1.12
IL-10	1411	0.03	90.6	1.55	7.21	0.89	0.24	1.67
TNF- α	1411	0.04	100	7.15	7.15	6.10	3.47	8.87
IL-5	1411	0.22	100	1.33	2.15	0.80	0.38	1.46
MCP-1	1411	0.09	100	74.20	86.59	59.66	42.90	86.04
TARC	1411	0.22	100	101.36	143.05	77.62	46.71	120.58
sFlt-1	1411	0.56	100	2056.99	1222.29	1778.93	1224.07	2523.61
VEGF-D	1411	2.53	100	737.46	369.45	659.24	484.94	907.59
CRP (mg/L)	1411	1.00	98.9	3.52	2.71	2.71	1.40	4.89
Th1	1411			13.28	21.20	9.67	5.75	14.75
Th2	1411			3.02	7.60	2.14	1.04	3.31
Th1:Th2	1411			8.23	12.63	5.16	3.04	8.48
Data are unlogged								

Table 3.

Correlations between covariates and birth outcomes

	Birth weight		Birth length		Head circumference		Gestational Age	
	r	P	r	P	r	P	r	P
Maternal age (yrs)	0.090	0.001	0.150	<0.001	0.048	0.076	-0.005	0.838
BMI (kg/m ²)	0.135	<0.001	0.129	<0.001	0.078	<0.001	0.067	0.017
Hollingshead SES	0.038	0.166	0.043	0.114	0.020	0.467	-0.012	0.656
Parity	0.082	0.002	0.132	<0.001	0.044	0.107	-0.018	0.501
Child sex	0.125	<0.001	0.109	<0.001	0.095	<0.001	-0.008	0.753
Gestational age (wks)	0.484	<0.001	0.363	<0.001	0.352	<0.001		
Significant correlations are bolded P<0.05								

Table 4.

Associations between maternal serum inflammatory markers and birth outcomes: Adjusted models¹

	Birth weight (kg) ¹			Birth length (cm) ¹			Head circumference (cm) ¹			Gestational Age ²		
	95% CI	β	P	95% CI	β	P	95% CI	β	P	95% CI	β	P
IFN- γ	-0.039, 0.012	-0.026	0.288	-0.240, 0.144	-0.013	0.626	-0.052, 0.129	0.022	0.402	-0.071, 0.104	0.010	0.708
IL-1 β	-0.084, 0.087	0.001	0.971	-0.691, 0.601	-0.004	0.892	-0.164, 0.446	0.023	0.365	-0.265, 0.326	0.006	0.841
IL-2	-0.077, 0.082	0.001	0.955	-0.393, 0.805	0.018	0.500	-0.116, 0.446	0.030	0.248	0.091, 0.635	0.073	0.009
IL-4	-0.184, 0.017	-0.039	0.103	-0.923, 0.688	-0.008	0.775	-0.315, 0.442	0.008	0.743	-0.186, 0.508	0.025	0.363
IL-6	-0.034, 0.062	0.014	0.557	-0.296, 0.435	0.010	0.708	-0.067, 0.276	0.031	0.232	-0.175, 0.156	-0.003	0.912
IL-10	-0.050, 0.029	-0.012	0.614	-0.537, 0.062	-0.041	0.121	-0.154, 0.129	-0.004	0.862	-0.259, 0.014	-0.049	0.079
TNF- α	-0.039, 0.037	-0.002	0.947	-0.195, 0.378	0.017	0.530	-0.059, 0.210	0.029	0.270	-0.225, 0.037	-0.039	0.160
IL-5	-0.087, 0.003	-0.045	0.067	-0.307, 0.373	0.005	0.848	-0.209, 0.11	-0.016	0.546	-0.090, 0.220	0.023	0.411
MCP-1	-0.020, 0.026	0.006	0.797	-0.059, 0.290	0.034	0.194	-0.122, 0.043	-0.024	0.349	0.021, 0.220	0.069	0.013
TARC	-0.030, 0.012	-0.020	0.403	-0.134, 0.179	0.008	0.776	-0.141, 0.007	-0.046	0.074	-0.032, 0.111	0.030	0.281
sFlt-1	-0.031, 0.052	0.012	0.617	-0.180, 0.448	0.023	0.403	-0.240, 0.055	-0.033	0.219	-0.229, 0.058	-0.034	0.244
VEGF-D	-0.101, -0.010	-0.058	0.017	-0.927, -0.243	-0.089	0.001	-0.314, 0.009	-0.049	0.063	-0.063, 0.250	0.033	0.241
CRP	-0.083, -0.007	-0.059	0.020	-0.285, 0.292	0.001	0.981	-0.227, 0.044	-0.036	0.186	-0.110, 0.153	0.009	0.750
Th1	-0.041, 0.024	-0.012	0.620	-0.213, 0.277	0.007	0.798	-0.050, 0.180	0.029	0.268	-0.132, 0.092	-0.010	0.731
Th2	-0.065, 0.009	-0.036	0.136	-0.384, 0.173	-0.020	0.457	-0.156, 0.106	-0.010	0.710	-0.167, 0.087	-0.017	0.525
Th1:Th2	-0.015, 0.045	0.023	0.338	-0.184, 0.269	0.010	0.714	-0.062, 0.151	0.021	0.413	-0.074, 0.133	0.016	0.572

All inflammatory markers were logged after adding a constant to all +1

¹Models are adjusted for child sex, maternal age, gestational age, SES, parity and maternal BMI;

²Model adjusted for child sex, maternal age, SES, parity and maternal BMI

Significant results are bolded P<0.05