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Correlation between maternal inflammatory markers and fetomaternal adiposity

Nadine Farah [a,*], Andrew E. Hogan [b], Norah O'Connor [a], Mairead M. Kennelly [a], Donal O'Shea [b], Michael J. Turner [a]

[a] UCD Centre for Human Reproduction, Coombe Women and Infants University Hospital, Dublin 8, Ireland
[b] Department of Endocrinology & Obesity Immunology Group, St. Vincent's University Hospital, Dublin, Ireland

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ABSTRACT

Outside pregnancy, both obesity and diabetes mellitus are associated with changes in inflammatory cytokines. Obesity in pregnancy may be complicated by gestational diabetes mellitus (GDM) and/or fetal macrosomia. The objective of this study was to determine the correlation between maternal cytokines and fetomaternal adiposity in the third trimester in women where the important confounding variable GDM had been excluded. Healthy women with a singleton pregnancy and a normal glucose tolerance test at 28 weeks gestation were enrolled at their convenience. Maternal cytokines were measured at 28 and 37 weeks gestation. Maternal adiposity was assessed indirectly by calculating the Body Mass Index (BMI), and directly by bioelectrical impedance analysis. Fetal adiposity was assessed by ultrasound measurement of fetal soft tissue markers and by birthweight at delivery. Of the 71 women studied, the mean maternal age and BMI were 29.1 years and 29.2 kg/m^2 respectively. Of the women studied 32 (45%) were obese. Of the cytokines, only maternal IL-6 and IL-8 correlated with maternal adiposity. Maternal TNF-α, IL-β, IL-6 and IL-8 levels did not correlate with either fetal body adiposity or birthweight. In this well characterised cohort of pregnant non-diabetic women in the third trimester of pregnancy we found that circulating maternal cytokines are associated with maternal adiposity but not with fetal adiposity.

1. Introduction

Maternal obesity based on Body Mass Index (BMI) $\geqslant$30.0 kg/m^2 is associated with increased pregnancy complications and its prevalence is increasing worldwide [1–3]. Recent research has highlighted that adipose tissue is not merely a passive repository for energy, but rather it is a highly active organ [4]. Outside pregnancy, obesity has been associated with a number of circulating markers of inflammation [5–7]. During pregnancy, increased inflammation has been associated with pre-eclampsia and gestational diabetes mellitus (GDM), both of which are complications associated epidemiologically with maternal obesity [8,9].

Cytokines are proteins and peptides secreted by cells, which modulate the intercellular actions of the cell. Cytokines are the result of enhanced leucocytic activity for immunosurveillance. They serve to protect the fetus, but an inflammatory response can sometimes be harmful. Cytokines are produced in all the tissues of the body. The placenta and membranes secrete a large number of pro and anti-inflammatory cytokines and chemokines [10,11]. Labour itself leads to a release of cytokines.

The inflammatory cytokines include IL-6, TNF-α, IL-8, and IL-β. IL-6 is a glycosylated protein molecule produced mainly by the TH2 lymphocytes, but also by monocytes and macrophages. IL-6 is the main physiological mediator of the acute phase reaction. It has been used as a diagnostic indicator of infections and inflammation. TNF-α is a non-glycosylated protein which is stimulated by bacteria and is mainly produced by the T cells, monocytes, macrophages and neutrophils. IL-8 is a non-glycosylated protein which is secreted by macrophages and monocytes. The main action of IL-8 is chemotaxis of neutrophils to adhere to endothelium. It is essential in fetal innate immunity as neutrophils are the first cells to appear at the site of an infection.

Maternal obesity, GDM and preeclampsia have each been shown to influence intrauterine growth [12,13]. There is, however, a dearth of studies which have examined the relationship between maternal cytokines and fetomaternal adiposity. Adipocytes in the fetus begin to develop at around 15 weeks gestation. The number and size both increase particularly in the third trimester when fetal fat increase from 5 to 15% [14].

The objective of this study was to examine the correlation between maternal cytokines in the third trimester with both maternal and fetal adiposity in a well characterised cohort where GDM had been excluded by a diagnostic glucose tolerance test (GTT) at 28 weeks gestation.

2. Materials and methods

This was a prospective nested cohort study involving women previously recruited as part of a larger study [15]. In the larger

* Corresponding author. Tel.: +353 1 4085760; fax: +353 1 4085786.
E-mail address: drnfarah@gmail.com (N. Farah).

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study women were enrolled at their convenience when they presented for their GTT at around 28 weeks gestation. To minimise confounding variables, the study was confined to white European women. Women with chronic medical problems that predispose to abnormal intrauterine growth were excluded, as well as cases of multiple pregnancy and known congenital fetal abnormalities. Women with evidence of recent infection were also excluded. Informed written consent was obtained.

All the women recruited had an early pregnancy ultrasound scan to confirm gestational age. In early pregnancy maternal weight and height were measured digitally in a standardised way, and the BMI was calculated. At recruitment, maternal body composition and weight were measured using multifrequency eight electrodes bioelectrical impedance analysis (BIA) (Tanita MC 180 MA, Tokyo, Japan). At the GTT, a serum sample was taken and within 1 h from collection the sample was centrifuged at 3500 rpm for 10 min at 4 °C. The supernatant was collected in 0.5 ml aliquots and immediately stored at −70 °C until assayed.

Fetal body composition was also evaluated by abdominal ultrasound by one operator (NF) using a transabdominal curved array transducer on an ALOKA prosoundα7. The fetal abdominal subcutaneous tissue was measured on the anterior abdominal wall in millimetres anterior to the margins of the ribs, using magnification at the level of the abdominal circumference [16]. The fetal midthigh muscle thickness was measured at the level of the femur diaphysis as the distance from the outer border of the femur to the inner edge of the subcutaneous layer. The fetal midthigh subcutaneous tissue measurement was calculated by subtracting the distance from the outer border of the femur to the outer border of the subcutaneous layer from the thigh muscle.

Women were asked to return between 36 and 39 weeks gestation for a follow up assessment. At this visit a serum blood sample was taken and centrifuged and then the serum was stored at −80 °C until assayed. Maternal body composition measurements were repeated and an ultrasound scan was performed by the same operator (NF) to measure fetal body composition. The clinical outcomes of the pregnancy were obtained from the medical records.

Serum cytokine concentrations of IL-β, IL-6, IL-8 and TNF-α, where investigated by multiplex immunoassay. All reagents and samples were brought to room temperature before use and all plasma samples were run in duplicate. MSD multiplex assays employ a sandwich immunoassay format where capture antibodies are coated in a patterned array, on the bottom of the wells of plate, these multiplex assays can be used to detect up to 10 analytes in cell supernatant, serum or tissue lysates. A seven point standard curve is generated on each plate and samples are interrogated with a lower level of detection of 0.6 pg/ml as per manufacturer's guidelines.

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc. Chicago, IL, USA). Relevant descriptive statistics (mean, standard deviation and percentages) were obtained for the study population. The paired sample *t*-test was used to detect changes in the parameters between 28 and 37 weeks gestation. Based on a sample size of 71, the analysis will have at least 80% power to detect a change in mean scores of 0.35 standard deviations at a level of 5% significance. The one-way analysis of variance (ANOVA) was used to detect differences between the groups. The relationship between maternal inflammatory markers and maternal and fetal parameters was investigated by use of Pearson correlation coefficients. The study was approved by the Hospital Research Ethics Committee.

3. Results

In the larger study, 259 women were recruited and attended their second assessment visit at 37 weeks gestation. Of those women, 28 had an abnormal OGTT and were excluded from further analysis [15]. From the resulting study population, 21 women with normal BMI, 18 overweight women, 17 women with class 1 obesity (BMI $\geqslant$ 30.0 and < 35.0 kg/m^2) and 15 women with class 2 and 3 obesity (BMI $\geqslant$ 35.0 kg/m^2) had inflammatory markers analysed at 28 and 37 weeks gestation. The characteristics of this study population are outlined in Table 1.

The mean values for the maternal inflammatory markers at both 28 and 37 weeks gestation are shown in Table 2. The increase in maternal TNF-α levels between 28 and 37 weeks gestation was statistically significant ($p = 0.009$). Although average maternal IL-β, IL-6 and IL-8 levels increased between 28 and 37 weeks gestation, this increase was not statistically significant. Maternal weight, fat mass and fat-free mass increased between 28 and 37 weeks gestation ($p < 0.001$) (Table 2). The fetal abdominal circumference (AC), abdominal subcutaneous tissue, midthigh muscle thickness and midthigh subcutaneous tissue ultrasound measurements also increased between 28 and 37 weeks gestation ($p < 0.001$) (Table 2).

At 28 weeks gestation, maternal IL-6 and IL-8 levels correlated with maternal fat mass ($r = 0.3$ and 0.3; $p = 0.005$ and 0.028 respectively). Maternal IL-β and TNF-α levels at 28 weeks gestation did not correlate with any of the maternal parameters.

At 37 weeks gestation, maternal IL-β, IL-8 and TNF-α did not correlate with any of the maternal parameters, but maternal IL-6 continued to correlate with maternal weight and fat mass ($r = 0.3$, and 0.3; $p = 0.011$ and 0.010 respectively). Table 3 outlines

Table 1
Characteristics of the study population ($n = 71$).

Maternal characteristics	Frequency (%)	Mean ± SD	Range
Age (years)		29.1 ± 4.8	20.0–41.0
Parity		0.8 ± 0.9	0–3
Early pregnancy BMI (kg/m^2)		29.2 ± 6.1	19.1–42.5
Prenatal smoking	16.9		
Newborn characteristics			
Gestational age at delivery (weeks)		40.3 ± 1.0	38.0–42.0
Birth weight (kg)		3.7 ± 0.5	2.7–4.8
Birth weight $\geqslant$ 4.5 kg	5.6		
Male sex	47.9		
Obstetric outcomes			
Hypertension			
Gestational hypertension	7.0		
Preeclampsia	4.2		
Induction rate	32.4		
Caesarean delivery	28.2		

BMI: Body Mass Index

Table 2
Mean maternal and fetal body composition and biomarkers values at 28 and 37 weeks gestation ($n = 71$).

Maternal	28 weeks	37 weeks
Weight (kg)	85.8 (17.1)[a]	90.2 (17.9)[a]
BMI (kg/m^2)	31.5 (6.0)[a]	33.2 (6.2)[a]
Fat mass (kg)	32.9 (10.9)[a]	34.4 (10.9)[a]
Fat-free mass (kg)	52.8 (6.6)[a]	55.7 (7.8)[a]
IL-β (pg/ml)	0.8 (0.6)	0.9 (0.7)
IL-6 (pg/ml)	1.8 (1.4)	2.0 (1.0)
IL-8 (pg/ml)	5.0 (1.8)	6.1 (2.9)
TNF-α (pg/ml)	7.3 (1.8)[b]	7.8 (1.9)[b]
Fetal		
Abdominal circumference (mm)	242.1 (1.4)[a]	331.5 (1.8)[a]
Abdominal subcutaneous tissue (mm)	3.4 (0.7)[a]	6.5 (1.6)[a]
Midthigh muscle thickness (mm)	8.0 (1.8)[a]	10.3 (2.8)[a]
Midthigh subcutaneous tissue (mm)	2.8 (0.8)[a]	4.9 (1.3)[a]

[a] $p < 0.001$.
[b] $p = 0.009$.

Table 3
Maternal biomarkers at 28 and 37 weeks gestation analysed by BMI category ($n = 71$).

At 28 weeks gestation	Normal BMI ($n = 21$)	Overweight ($n = 18$)	Class 1 obese ($n = 33$)	Class 2 and 3 obese ($n = 15$)
IL-β (pg/ml)	0.6 (0.5)	0.9 (0.9)	1.0 (0.5)	1.0 (0.6)
IL-6 (pg/ml)	1.3 (0.7)	1.9 (1.1)	1.9 (0.8)	2.4 (2.6)
IL-8 (pg/ml)	4.9 (1.8)	4.8 (1.3)	5.7 (2.4)	7.9 (12.1)
TNF-α (pg/ml)	7.3 (1.8)	7.3 (1.9)	7.2 (1.7)	7.2 (1.7)
At 37 weeks gestation				
IL-β (pg/ml)	0.9 (0.8)	0.8 (0.5)	1.1 (0.8)	0.8 (0.4)
IL-6 (pg/ml)	1.5 (0.7)	2.0 (1.1)	2.2 (0.9)	2.2 (1.0)
IL-8 (pg/ml)	5.7 (2.6)	6.0 (3.6)	6.9 (2.7)	6.0 (2.8)
TNF-α (pg/ml)	8.0 (2.2)	7.5 (2.2)	7.7 (1.8)	7.9 (1.5)

the mean values for the maternal inflammatory markers at both 28 and 37 weeks gestation analysed by BMI category. Maternal IL-β, IL-6, IL-8 and TNF-α levels did not correlate with any of the fetal body composition parameters at either 28 or 37 weeks gestation and nor did they correlate with birthweight.

4. Discussion

Several studies have firmly established the strong association between obesity and elevated inflammatory markers, leading to the recognition of obesity as a state of chronic low grade inflammation [17–19]. Maternal obesity is associated with increased risk of a variety of adverse outcomes [3]. In pregnancy, a few studies to date suggest that obesity is associated with inflammation. We found that maternal IL-6 and IL-8 levels correlate positively with maternal adiposity in the third trimester of pregnancy independently of GDM.

A study conducted in the United Kingdom on 47 women in the third trimester of pregnancy also showed that pregnant obese women have higher CRP and IL-6 levels compared to lean pregnant women [7]. However, the study does not report on how the maternal BMI was calculated, whether the women had a glucose tolerance test in the pregnancy and the gestation at which the inflammatory markers were assessed.

Although TNF-α levels are elevated in non-pregnant obese adults, we found that TNF-α levels do not correlate with maternal adiposity [20,21]. Our findings are similar to those by a cross-sectional study where 80 women had their serum inflammatory markers measured in the second trimester of pregnancy and by also another study where 53 lean and 68 obese women had their inflammatory markers measured at term before labour [22,23].

We found that maternal TNF-α levels increase between 28 and 37 weeks gestation. A longitudinal American study where fifteen women had body composition measurements on three occasions: pregravid and during both early (12–14 weeks) and late (34–36 weeks) pregnancy also found that maternal TNF-α levels increase in late pregnancy [24]. Studies have shown the placenta to be an important source of TNF-α with greatest production rates evident in late gestation which explains the increase in TNF-α levels [25]. Maternal IL-β, IL-6 and IL-8 levels did not change between 28 and 37 weeks gestation. A longitudinal study carried out in Switzerland on 38 pregnant multiethnic women also found that IL-β, IL-6 and IL-8 levels did not change in pregnancy and remained consistently low [26]. They found that IL-β levels increased shortly before labour, while IL-6 and IL-8 levels rose in labour.

The impact of maternal obesity on the offspring is substantial. In the long-term there is increasing observational evidence suggestive of in utero programming of offspring in an adverse maternal environment. Epidemiological evidence has shown that babies born to obese mothers are more likely to be obese in childhood and adulthood and have increased risk of cardiovascular disease in later life [27–29]. We found that maternal IL-β, Il-6, IL-8 and TNF-α levels do not correlate with fetal adiposity in women with a normal GTT. An American study found an association between increased fetal adiposity and maternal IL-6 [30]. In this study, 18 multiethnic women with a singleton pregnancy were recruited and neonatal anthropometry was carried out within 24 h of delivery. The mean BMI of the study population was similar to that in our study but, in the American study women with GDM were included in the analysis.

We acknowledge that our study has a number of weaknesses. Recruitment was by convenience rather than consecutive. As obesity was an indication for a screening GTT, the number of obese women and, in particular, the number of women with moderate to severe obesity is overrepresented compared with the general population [3]. This overrepresentation, however, did facilitate comparison with women in the normal BMI category. While the numbers studied appear small they do compare favourably with the numbers in previous studies and, in particular, the proportion of women in previous studies with moderate to severe obesity.

An advantage of our study is that it is prospective and the patient population is well characterised. Specifically, women with existing hypertension, prepregnancy or GDM have been excluded. The study was confined to white European women with a singleton pregnancy which avoids the confounding variables of ethnicity and multiple pregnancies. All women had their pregnancy dated in the first trimester which is important because gestational age is important for timing the GTT and is a key determinant of fetal adiposity measurements and birthweight. The calculation of BMI was based on accurate measurement of weight and height and not on selfreporting which has been shown to result in the miscategorisation of BMI values [31].

The study also included the novel use of fetal soft tissue markers to assess fetal adiposity and the use of advanced bioelectrical impedance analysis to assess maternal body composition. Recent studies have shown that BIA in pregnancy is reproducible and correlates well with biomarkers of maternal adiposity and with clinical outcomes [32–34].

Our results show that, as in nonpregnant obese adults, obesity in pregnancy is associated with an inflammatory up-regulation. However, in women with a normal GTT we found no correlation between maternal inflammatory markers and fetal adiposity. Further studies are required to confirm whether fetal cytokines are associated with fetal adiposity, in women where GDM has been excluded.

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