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Retinol-binding protein 4 levels are related to maternal triglyceride levels

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Abstract

Aim of the study: *To detect whether there is an association between interleukin 8 (IL-8), IL-17, retinol binding protein 4 (RBP4) and birth weight and maternal biochemical parameters.*

Material and methods: *We obtained simultaneous maternal and cord blood from 77 patients. Blood for RBP4, IL-8 and IL-17 was stored.*

Results: *There was no statistically significant correlation between maternal and cord blood RBP4, IL-8, IL-17 levels and birth weight percentiles. There was a statistically significant correlation between maternal RBP4 and maternal triglyceride and VLDL levels ($r = 0.239$; $p = 0.038$ and $r = 0.278$; $p = 0.038$), between maternal IL-17 and maternal LDL levels ($r = 0.253$; $p = 0.031$). There was a positive relation between RBP4 and maternal blood glucose in the bottom quartile group (< 25th percentile) ($r = 0.612$; $p = 0.015$).*

Conclusion: *Maternal RBP4 levels were related to maternal triglyceride levels. There was no association between RBP4, IL-8, IL-17 and birth weight.*

Key words: *retinol-binding protein 4, interleukin 8, interleukin 17, birth weight, pregnancy.*

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Introduction

Implantation is a controlled inflammatory process. An exaggerated response in this inflammatory process may lead to defective placentation, which results in "Great Obstetrical Syndromes" including fetal growth restriction and preeclampsia [1]. Many studies focused on specific inflammatory markers that would aid to define abnormalities in the inflammatory process of implantation and subsequent placentation.

Retinol binding protein 4 (RBP4) is an adipocytokine, and is also expressed by macrophages under the regulation of inflammatory stimuli [2]. Retinol binding protein 4 is a specific blood-carrier of retinol, and is overexpressed in adipose tissue of insulin-resistant and diabetic patients [3].

Subsequent reports regarding the association between maternal plasma RBP4 levels and gestational diabetes mellitus presented controversial results [4-6]. Retinol binding protein 4 was also reported to be expressed by macrophages and to be regulated by inflammatory stimuli [2]. Changes in RBP4 levels correlated with those of inflammatory markers [7]. Increased levels of the chemokine interleukin 8 (IL-8) was reported to be associated with fetal growth restriction in preeclamptic pregnancies [8] and IL-17 was reported as a proinflammatory cytokine that induced the secretion of IL-8 [9].

Previous studies comparing RBP4 levels with birth weight reported conflicting results [10-12]. We hypothesized that abnormalities in maternal RBP4 levels and the associated proinflammatory state, controlled by IL-8 and IL-17 levels, might affect birth weight. We compared RBP4 levels with maternal biochemical parameters. We also measured cord blood RBP4, IL-8 and IL-17 levels to control the related associations.

Material and methods

The study was designed as a prospective clinical study. The study protocol was designed according to the Decla-

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ration of Helsinki. The individuals who had routine follow-up for pregnancy at the İstanbul Bilim University Department of Gynecology and Obstetrics between January 2010 and January 2012 were recruited for the study. The pregnants were fully informed about the details of the study. Informed consent was obtained from those who accepted to join the study.

Singleton pregnants who had C-section after the 36th gestational week were included in the study. Exclusion criteria were:

- multifetal gestation,
- presence of ruptured membranes before delivery,
- presence of maternal diseases such as gestational diabetes mellitus, gastrointestinal malabsorptive diseases, hypertensive diseases of pregnancy, suspicion of infectious diseases,
- diagnosis of fetal anomalies (abnormal fetal karyotype, congenital malformations),
- fetal distress,
- presence of meconium in the amniotic fluid,
- unwillingness to join the study.

Ten milliliters of maternal blood sample was drawn from the maternal peripheral vein. Maternal blood samples were equally divided into two dry vacutainer tubes. One tube was used immediately after sampling for detection of blood glucose, insulin, C-reactive protein (CRP), triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), and total cholesterol, whereas the other tube was stored at –80°C until biochemical analysis for RBP4, IL-8 and IL-17. Five milliliters of cord blood sample was obtained from the placental side of the cord after transection of the cord during C-section, was kept in a dry vacutainer tube and was stored at –80°C until biochemical analysis for RBP4, IL-8 and IL-17.

The concentrations of RBP4, IL-17, and IL-8 were determined by ELISA (enzyme-linked immunosorbent assay) utilizing RBP4 kit (Assaypro, Belgium), IL-17 kit (Cytoscreen, Nivelles, Belgium), and IL-8 kit (Invitrogen, Camarillo, CA), respectively. Insulin resistance was measured by homeostasis model assessment (HOMA) of insulin resistance using the following formula: HOMA-IR = fasting insulin (mU/ml) × fasting glucose (mg/dl)/405. Gestational age was measured according to the last menstrual period or the crown-rump length within the first trimester.

Statistical analysis was made using NCSS (Number Cruncher Statistical System) 2007 and PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA). Data showing demographic parameters were presented as mean values and standard deviation. The relationships between quantitative variables were studied by Spearman's correlation analysis. Kruskal-Wallis test was used for the comparison of continuous variables among groups. Multivariate regression analysis was employed to determine factors that correlated with maternal and cord blood variants. A p-value of less than 0.05 was considered to be statistically significant.

Table 1. Demographic features of the patients and maternal serum concentrations of the metabolic parameters

Parameter	Range	Mean ± SD	Median
Maternal age (years)	23-41	31.5 ±4	31
First trimester BMI (kg/m^2)	18-40	23.3 ±3.8	22.5
Birth weight (grams)	1950-4300	3420 ±461.7	3420
Gestational week at delivery (weeks)	36-41.3	38.9 ±1.4	38.86
Triglyceride (mg/dl)	10-544	259.7 ±97.9	251.5
Insulin (mU/ml)	0.6-60.5	9.7 ±7.1	7.1
CRP (mg/l)	0.01-1.75	0.47 ±0.4	0.34
Cholesterol (mg/dl)	37-343	233.9 ±64.3	245
HDL (mg/dl)	19-155	64.5 ±22.7	65.5
LDL (mg/dl)	12-238	117.4 ±46.2	114
VLDL (mg/dl)	2-102	51.7 ±19.1	50
HOMA-IR	0.1-21.4	1.99 ±2.9	1.2

Table 2. Levels of maternal and cord blood RBP4, IL-8 and IL-17

	Range	Mean ±SD	Median
Maternal RBP4	6.00-183.2	80.7 ±35.4	81.6
Maternal IL-8	0.02-12.5	0.9 ±1.7	0.39
Maternal IL-17	0.5-129.7	13.3 ±19.5	6.10
Cord RBP4	6.0-134.4	61.9 ±26.6	61.8
Cord IL-8	0.02-20.6	1.5 ±3.4	0.4
Cord IL-17	1.2-82.9	18.6 ±16.7	14.9

Results

The total number of subjects included in the study was 77. Demographic data and results of the biochemical parameters showing maternal metabolic status are presented in Table 1. The levels of RBP4, IL-8 and IL-17 in maternal and cord blood are presented in Table 2. Table 3 illustrates the comparison of maternal and cord blood levels of RBP4, IL-8 and IL-17 with birth weight percentiles. There was no statistically significant correlation between maternal and cord blood RBP4, IL-8, IL-17 levels and birth weight percentiles. Cord blood IL-8 and maternal IL-17 levels were higher in the bottom quartile group (< 25th percentile) but did not reach statistically significant levels. We also checked the correlation between birth weight and maternal and cord

Table 3. The relationship between birth weight percentiles and maternal and cord blood RBP4, IL-8 and IL-17 levels

	Birth weight			*p*
	< 25th percentile	25th-75th percentile	> 75th percentile	
	Mean ±SD (median)	Mean ±SD (median)	Mean ±SD (median)	
Maternal RBP4	83.7 ±20.2 (83.1)	79.6 ±37.3 (80.8)	81.8 ±43.9 (80.4)	0.816
Maternal IL-8	1.6 ±3.1 (0.6)	0.7 ±1.1 (0.4)	0.7 ±1.4 (0.4)	0.205
Maternal IL-17	18.4 ±21.9 (10.8)	12.2 ±20.3 (4.3)	11.2 ±11.7 (6.5)	0.144
Cord RBP4	59.6 ±17.8 (58.7)	62.8 ±28.3 (61.4)	61.4 ±30.5 (64)	0.837
Cord IL-8	2.6 ±4.8 (1.3)	1.3 ±3.2 (0.4)	0.9 ±1.2 (0.5)	0.117
Cord IL-17	20.8 ±22.2 (12.4)	18.2 ±14.8 (14.5)	17.6 ±17.5 (18.1)	0.957

Kruskal Wallis Test

Table 4. Correlation of maternal blood levels of RBP4, IL-8 and IL-17 with maternal metabolic parameters

n = 77	RBP4		IL-8		IL-17	
	r	*p*	*r*	*p*	*r*	*p*
Triglyceride	0.239	0.038*	–0.144	0.214	0.003	0.980
Insulin	0.099	0.391	–0.012	0.915	–0.215	0.060
TSH	–0.002	0.988	–0.045	0.766	–0.086	0.572
CRP	0.102	0.377	–0.086	0.459	0.022	0.847
Blood glucose	0.097	0.403	–0.107	0.353	0.092	0.424
Cholesterol	0.005	0.969	0.055	0.633	0.138	0.230
HDL	–0.035	0.766	0.094	0.421	0.099	0.397
LDL	–0.155	0.191	0.084	0.479	0.253	0.031*
VLDL	0.278	0.038*	–0.076	0.580	–0.077	0.572
HOMA-IR	0.103	0.372	–0.012	0.920	–0.014	0.236

r: Spearman's Correlation Coefficient
**p < 0.05*

blood levels of RBP4, IL-8 and IL-17. There was no statistically significant relationship. Maternal RBP4 levels were in correlation with cord blood RBP4 levels ($r = 0.616$; $p = 0.001$). Maternal IL-8 levels were in correlation with cord blood IL-8 levels ($r = 0.555$; $p = 0.001$). Maternal IL-17 levels were in correlation with cord blood IL-17 levels ($r = 0.581$; $p = 0.001$).

Maternal biochemical parameters were compared with maternal levels of RBP4, IL-8 and IL-17 and the results are presented in Table 4. There was a statistically significant correlation between maternal RBP4 levels and maternal triglyceride and VLDL levels ($r = 0.239$; $p = 0.038$ and $r = 0.278$; $p = 0.038$). There was also a statistically significant correlation between maternal IL-17 levels and maternal LDL levels ($r = 0.253$; $p = 0.031$). When the maternal biochemical parameters were compared with RBP4, IL-8 and IL-17 according to percentile groups, the only positive correlation was observed between maternal RBP4 and glucose in the bottom quartile group (< 25th percentile) ($r = 0.612$; $p = 0.015$).

There was no correlation between maternal BMI and maternal biochemical parameters (RBP4 $r = 0.047$, $p = 0.687$; IL-8 $r = –0.036$, $p = 0.757$; IL-17 $r = –0.146$, $p = 0.208$; TG $r = –0.092$, $p = 0.435$; insulin $r = 0.221$, $p = 0.055$; TSH $r = 0.207$, $p = 0.168$; CRP $r = 0.037$, $p = 0.753$; glucose $r = –0.057$, $p = 0.623$; HDL $r = –0.115$, $p = 0.327$; LDL $r = –0.184$, $p = 0.122$; VLDL $r = –0.154$, $p = 0.263$; HOMA-IR $r = 0.181$, $p = 0.117$).

Discussion

In our study we attempted to determine the relationship between the controlled inflammatory process of implanta-

tion and fetal growth restriction [1]. A shift toward IL-17 production was reported in preeclampsia [13, 14] and the same process was suggested to play a role in acute transplant rejection [15]. Interleukin 17 was suggested to be involved in recurrent pregnancy loss [13, 16]. Previously, increased levels of IL-8 were reported in cord blood of preeclamptic pregnancies with fetal growth restriction [8, 17]. In our study, slightly increased levels of maternal IL-17 and IL-8 in the group at the lowest birth weight quartile suggest an association between IL-17 and IL-8 and defective placentation. Many other studies tried to find evidence of the connection between cytokines and fetal growth. Gene polymorphisms associated with decreased levels of anti-inflammatory cytokines IL-4 and IL-10 were associated with the delivery of SGA infants [18]. Infants born SGA were also reported to have higher cord blood IL-6, TNF-α and CRP levels [19]. On the contrary, in another study TNF-α and IL-6 levels were not related to delivery of SGA infants, but to preterm delivery [18]. Cord levels of IL-6 were reported to decrease with the decreasing birth weight [20]. Levels of IL-8, IL-6, TNF-α and CRP were found to be increased in preeclamptic pregnancies [17]. All of these findings were suggestive of an association between interleukins and placentation defects.

Interleukin 8 was suggested to regulate angiogenesis in early pregnancy [21] and increased levels were reported in the decidual tissue [22]. Interleukin 8 was also shown to promote the migration of first trimester trophoblasts [23]. The maternal and fetal low grade inflammatory state seems to affect the implantation process. Future studies investigating the relationship between IL-8 and IL-17 levels and birth weight in larger study groups may reach statistically significant results. Cord blood levels of IL-8 were higher in preeclamptic and normal pregnancies when compared to maternal blood levels [8]. In our study, both IL-8 and IL-17 levels were higher in cord blood when compared to maternal blood levels, too. Previously, most of the proinflammatory cytokines and RBP4 were shown not to cross the placenta [24, 25].

Previously a correlation between RBP4 levels and markers of inflammation in children was shown [7]. Another cytokine, adiponectin, was shown to decrease IL-8 and IL-6 secretion from endometrial stromal cells [26]. We searched for an association between RBP4 and the inflammatory markers, IL-8 and IL-17. Our study demonstrated that maternal and cord blood levels of RBP4 had no association with fetal birth weight and with IL-8 and IL-17 levels. Previously, high levels of cord blood RBP4 were suggested to be associated with the delivery of LGA/SGA neonates [10] but another study opposed these findings [12]. Giacomozzi *et al*. suggested an association between SGA infants and cord blood RBP4 levels [11], RBP4 levels were suggested to decrease in SGA infants independent of insulin resistance markers [11]. We did not control the fetal markers of insulin resistance in cord blood, but the maternal markers of insulin resistance were not correlated with RBP4 levels. The only correlation was observed between maternal glucose and RBP4 levels in fetuses of the bottom quartile. Previously plasma RBP4 levels were found to increase with increasing TG levels [6, 27, 28]. On the contrary, in a recent study no association was reported between maternal RBP4 levels and prepregnancy BMI, birth weight, parameters of insulin resistance and TG levels [29]. Higher maternal plasma levels of RBP4 were reported to be associated with larger neonates both in women with and without gestational diabetes mellitus [5], high maternal RBP4 concentrations were attributed to maternal glucose metabolism. In that study, overweight mothers had higher RBP4 levels and this suggested an association related to maternal adipose tissue mass, rather than glucose metabolism. In our study, maternal RBP4 levels correlated with TG levels, an indicator of maternal adipose tissue mass, but did not correlate with the markers of insulin resistance, this was in accordance with a previous study [28].

Circulating RBP4 levels were influenced by the retinol and iron status of the subjects [7]. Nearly all of our patients received multivitamin supplementations containing vitamin A and iron during pregnancy. We did not control the serum levels of these two metabolites and this might have affected the results. Another limitation of the study was the small number of fetuses in the bottom quartile. Exclusion of patients delivering vaginally and those in active labor might have helped us to escape from the confounding factors related to maternal and fetal stress.

In conclusion, the exaggerated inflammatory response in pregnancy may be associated with underdevelopment of the fetus. Maternal RBP4 levels are related to maternal TG levels. There was no association between birth weight and IL-8, IL-17 and RBP4 levels.

Authors declare non conflicts of interest.

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