THE JOURNAL OF
Obstetrics and Gynaecology Research

The official Journal of Asia and Oceania Federation of Obstetrics and Gynaecology
Japan Society of Obstetrics and Gynecology

doi:10.1111/jog.12499 J. Obstet. Gynaecol. Res. Vol. 41, No. 1: 55–61, January 2015

Early second trimester retinol-binding protein-4 values in cases with or without gestational diabetes mellitus risk factors: A cross-sectional study

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Abstract

Aim: Retinol-binding protein-4 (RBP-4) has been correlated with different degrees of insulin resistance including gestational diabetes mellitus (GDM). Presence of risk factors for GDM is an indication for early screening. We studied RBP-4 values in the early second trimester of pregnancy in pregnant subjects with or without GDM risk factors and compared the results by routine GDM screening methodology.

Methods: Seventy-nine patients with at least one GDM risk factor and 46 patients without any GDM risk factors were enrolled in the cross-sectional study as risk and control groups, respectively. In the early second trimester, RBP-4 values were measured, in addition to fasting plasma glucose and 50-g glucose challenge test in all subjects.

Results: The RBP-4 values in 16–18th weeks of pregnancy were not significantly different between risk and control groups (95.3 ± 20.1 vs 103.1 ± 24.4 μg/mL, respectively; $P = 0.055$) although fasting plasma glucose levels and 50-g glucose challenge test results were higher in the risk group than the control group (75.3 vs 69.3 mg/dL and 112.4 vs 97.5 mg/dL, respectively; $P < 0.05$).

Conclusion: Presence of GDM risk factors does not have an impact on early second trimester RBP-4 values in pregnant subjects.

Key words: early second trimester, gestational diabetes mellitus, retinol-binding protein 4.

Introduction

Gestational diabetes mellitus (GDM) complicates 1.7–11% of pregnancies[1] and is still an important health problem associated with fetal macrosomia, shoulder dystocia, stillbirth, hypocalcemia, hypoglycemia, hyperbilirubinemia, respiratory distress syndrome, and increased maternal and fetal diabetes mellitus risk. Identifying women with GDM is important to improve the outcomes. Although the criteria for screening and diagnosis of GDM is controversial and an international agreement is lacking, the American Diabetes Association and the American College of Obstetrics and Gynecologists recommend routine screening for GDM in pregnancy.[2–4]

Early screening of all pregnant women will help to identify GDM cases that will lead to earlier interventions and may decrease associated morbidities. The association between different serum markers measured early in pregnancy, in the first or early second trimester, and GDM were reported previously.[5–7] Placental diabetogenic hormones cause insulin resistance (IR) and hyperinsulinemia which predispose women to development of diabetes mellitus in pregnancy. In

Received: August 27 2013.
Accepted: May 7 2014.
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Conflict of Interests: The authors declare that no actual or potential conflict of interest in relation to this article exists.

uncomplicated pregnancies[8] and pregnancies with GDM,[9] Retinol-binding protein-4 (RBP-4) increases significantly through gestation parallel to a decrease in insulin sensitivity.

Retinol-binding protein-4 is a 21-kDa polypeptide, an adipokine, secreted from hepatocytes and adipocytes. The main function of RBP-4 is to bind and transport vitamin A (retinol).[10] Recent research indicated that RBP-4 levels are correlated with IR.[11,12] Although higher RBP-4 values have been reported in women with GDM, comparison of early second trimester RBP-4 values with early second trimester 50-g glucose challenge test (GCT) results, in cases with and without GDM risk factors have not been reported yet.

Other than pregnancy and GDM,[13] RBP-4 has been shown to be upregulated in various insulin-resistant states such as obesity, type 2 diabetes mellitus[12] and metabolic syndrome.[14] However, whether RBP-4 is the promoter or the result of the IR or a molecule correlated with different parameters of metabolic syndrome is not clear yet.[15] The elevated RBP-4 levels in type 2 diabetes mellitus was suggested as a secondary and predominantly non-genetic phenomenon. The authors reported that plasma RBP-4 plays a minor role in development of IR in humans.[15]

This study was designed to evaluate whether there is a difference in RBP-4 levels in the early second trimester between pregnant women with and those without risk factors for GDM.

Methods

Study group

This is a cross-sectional study consisting of data from 125 pregnant subjects admitted to an outpatient clinic of department of obstetrics and gynecology of a university hospital between January 2007–January 2009 ($n = 630$). The recruitment of the patients to the study was stopped at the 18th month and the study ended when all the cases delivered. The patients admitted for the routine visit at 16–18 weeks of pregnancy were first evaluated ($n = 410$) for the study. Second, patients who were informed verbally about the planned study, agreed to participate and provided written consent were included in the study ($n = 164$). The 164 pregnant patients in their routine 16–18th week visit, were asked about the gestational diabetes risk factors (pre-pregnancy body mass index [BMI], >25; GDM history in previous pregnancy; type 2 diabetes mellitus in first-degree relatives; previously giving birth to a >4000-g infant; poor obstetric history such as missed abortus, malformed infant, polyhydramnios, stillbirth or preterm birth).[3,16] Twenty of the patients ($n = 10$ in the risk group and $n = 10$ in the control group; total, $n = 20$) who did not fulfill the inclusion criteria were excluded. As a result, 144 patients who gave written informed consent and fulfilled the inclusion criteria were eligible and were incorporated either in the risk group ($n = 83$) in the presence of any risk factors or control group ($n = 61$) in absence of any. Patients who did not attend their visits and who did not give birth in the same center were excluded (risk group, $n = 4$ vs control group, $n = 15$) leading to final data of 125 patients (risk group, $n = 79$ vs control group, $n = 46$) who were eligible for statistical analysis. Patients with any additional medical conditions (e.g. hypertension, thyroid disorders, pre-existing diabetes mellitus) were excluded from the study. The study was approved by the local ethics committee and informed consent was taken from the participants.

Study design

In the same visit, fasting plasma glucose (FPG), 50-g GCT and RBP-4 values were measured in both groups of patients. In case of FPG of more than 95 mg/dL or 50-g GCT of more than 140 mg/dL, 100-g oral glucose tolerance test (OGTT) was performed with the reference values of Carpenter and Coustan.[16] At 24–28 weeks of pregnancy, GDM screening was performed in all patients. Similarly, FPG was measured and 50-g GCT was also performed in all of the subjects. In cases with the previously mentioned results above cut-off values, 100-g OGTT was performed. In case of a high 50-g GCT, the patient was put on a diet because abnormal 50-g GCT on its own has been associated with adverse pregnancy outcomes.[17–19] Gestational week at birth, route of delivery (vaginal or cesarean), sex and birthweight of newborn and 1- and 5-min Apgar scores were recorded.

Laboratory

Blood samples were taken into empty separator tubes and let to clot for 30 min at room temperature and centrifuged for 20 min at 1000 *g*. Serum samples were stored at –80°C until assayed for RBP-4 analysis. RBP-4 measurement was performed by Human RBP-4 Competitive ELISA Kit (Invitrogen, San Diego, CA, USA) and enzyme-linked immunosorbent assay (ELISA). The sensitivity was 1 ng/mL and intra- and inter-assay coefficients of variation were 2.6–9.2% and 3.4–10.2%,

Table 1 Demographic variables of the risk and control groups

Variable	Control group ($n = 46$) Mean (range)	Risk group ($n = 79$) Mean (range)	P-value
Age (years) (±SD)	23.3 ± 3.5	28.7 ± 4.5	<0.001*
Body mass index (kg/m^2) (±SD)	23.5 ± 0.9	25.1 ± 4.0	0.076
Gravidity	1 (1–3)	2 (1–8)	<0.001*
Parity	0 (0–2)	1 (0–3)	<0.001*
Abortus	0 (0–1)	0 (0–6)	0.007*
D&C	0 (0–1)	0 (0–2)	0.308
Previous live birth	0 (0–2)	0 (0–2)	<0.001*

*$P < 0.05$. D&C, dilatation and curettage; SD, standard deviation.

respectively. All the blood samples were studied in duplicate according to the manufacturers' instructions.

Statistical analyses

Data analysis was performed by using SPSS for Windows version 11.5 (SPSS, Chicago, IL, USA). Whether the distributions of continuous variables were normal or not was determined by Shapiro–Wilk test. Levene's test was used for the evaluation of homogeneity of variances. Data were shown as mean ± standard deviation or median (range), where applicable.

While the mean differences between control and risk groups were compared by using Student's t-test; otherwise, the Mann–Whitney U-test was applied for comparisons of the median values. Nominal data were analyzed by Pearson's χ^2-test. Degrees of associations between continuous variables were determined by Spearman's rank correlation coefficient analysis.

Whether RBP-4 measurements had a statistically significant effect on GDM risk or not was evaluated by multiple logistic regression analysis after adjustment for both age and BMI. Any variable whose univariate test had a P-value of less than 0.25 was accepted as a candidate for the multivariate model along with all variables of known clinical importance. Odds ratios and 95% confidence intervals for each independent variable were also calculated.

A P-value less than 0.05 was considered statistically significant.

Results

One hundred and twenty-five pregnant patients who completed antenatal follow-up and gave birth in the same hospital were included in the study. The risk group consisted of pregnant subjects with at least one GDM risk factor ($n = 79$) while subjects without any GDM risk factors constituted the control group ($n = 46$). The demographic characteristics of participants are given in Table 1. As expected; the mean age, gravidity, parity, abortus and previous live birth rates were statistically higher in the risk group ($P < 0.05$). The most common risk factor was maternal age over 25 years ($n = 64$, 81%) while first-degree relative and history of missed abortus were the following most frequent ones (Table 2). Distribution of FPG, 50-g GCT and 100-g OGTT results in risk and control groups are shown in Table 3.

Table 2 Distribution of risk factors in risk group

Risk factor	Risk group ($n = 79$) n (%)
Previous gestational diabetes mellitus	1 (1.2%)
Maternal age (years)	64 (81.0%)
First-degree relative with diabetes mellitus	20 (25.3%)
Previous birth of >4000 g	6 (7.6%)
Missed abortus	19 (24%)
Polyhydramnios history	2 (2.5%)
Stillbirth history	2 (2.5%)
Preterm birth history	4 (5.0%)
Child with congenital abnormality	2 (2.5%)
Body mass index, >25 kg/m^2	30/60 (50.0%)

Gestational week at birth was higher in the control group when compared to the risk group (39.4 ± 1.4 vs 38.6 ± 1.9 weeks, respectively; $P = 0.033$). Route of delivery (vaginal or cesarean), sex, birthweight and 1- and 5-min Apgar scores of the newborns did not differ significantly between the two groups ($P > 0.05$) (Table 4). As expected, 16–18th week FPG and 50-g GCT values were significantly higher in the risk group than the control group (75.3 vs 69.3 mg/dL and 112.4 vs 97.5 mg/dL, respectively; $P < 0.05$). But RBP-4 values did not differ significantly between the risk and control groups (95.3 ± 20.1 μg/mL vs

Table 3 Distribution of results of 50-g GCT and 100-g OGTT in control and risk groups

Variable	Control group ($n = 46$)	Risk group ($n = 79$)
16–18th week of gestation		
50-g GCT (N)†	43 (93.5%)	60 (75.9%)
50-g GCT (H)‡/100-g OGTT (N)§	1 (2.2%)	16 (20.3%)
50-g GCT (H)‡/100-g OGTT (1)¶	1 (2.2%)	2 (2.5%)
50-g GCT (H)‡/100-g OGTT (2)††	1 (2.2%)	1 (1.3%)
24–28th week of gestation		
50-g GCT (N)†	45 (97.8%)	68 (86.1%)
50-g GCT (H)‡/100-g OGTT (N)§		4 (5.1%)
50-g GCT (H)‡/100-g OGTT (1)¶	1 (2.2%)	6 (7.6%)
50-g GCT (H)‡/100-g OGTT (2)††		1 (1.3%)

†50-g GCT result normal (<140 mg/dL). ‡50-g GCT result higher than normal. §100-g OGTT results are within normal range. ¶100-g OGTT with only one abnormal value (glucose intolerance). ††100-g OGTT with two abnormal values (e.g. gestational diabetes mellitus). GCT, glucose challenge test; H, high; N, normal; OGTT, oral glucose tolerance test.

Table 4 Characteristics and laboratory results of the risk and control groups

Variables	Control group ($n = 46$)	Risk group ($n = 79$)	*P*-value
Week of birth	39.4 ± 1.4	38.6 ± 1.9	0.033*
Route of delivery			0.113
Vaginal delivery	10 (23.8%)	29 (38.2%)	
Cesarean section	32 (76.2%)	47 (61.8%)	
Sex (neonate)			0.917
Female	19 (46.3%)	34 (45.3%)	
Male	22 (53.7%)	41 (54.7%)	
Birthweight (g)	3240 (2144–4029)	3280 (2000–4305)	0.546
1-min Apgar	8 (6–10)	8 (6–10)	0.963
5-min Apgar	10 (9–10)	10 (8–10)	0.782
16–18th week			
FPG (mg/dL)	69.3 ± 8.4	75.3 ± 14.6	0.006*
50-g GCT(mg/dL)	97.5 ± 26.6	112.4 ± 31.2	0.012*
RBP-4 (μg/mL)	103.1 ± 24.4	95.3 ± 20.1	0.055
24–28th week			
FPG (mg/dL)	68.6 ± 8.8	78.4 ± 36.5	0.005*
50-g GCT (mg/dL)	97.3 ± 21.8	116.9 ± 42.9	<0.001*

*$P < 0.05$. FPG, fasting plasma glucose; GCT, glucose challenge test; RBP-4, retinol-binding protein-4.

103.1 ± 24.4 μg/mL respectively; $P = 0.055$). Also, RBP-4 levels in patients with more than one risk factors were not significantly different than in the control group (96.7 ± 22.6 μg/mL vs 93.3 ± 16.2 μg/mL; $P = 0.468$). Additionally multiple logistic regression analysis revealed that RBP-4 values were not statistically significantly different between the risk and control groups after adjustment for age ($P = 0.354$). In a similar manner, FPG and 50-g GCT values were statistically significantly higher in the risk group than in the control group in routine GDM screening weeks that are at 24–28th weeks of gestation (78.4 vs 68.6 mg/dL and 116.9 vs 97.3 mg/dL, respectively; $P < 0.05$). Student's *t*-test revealed that the only risk factor associated with RBP-4 was maternal age. Maternal age over 25 years in the risk group was related with lower RBP-4 values (102 ± 24.5 vs 93 ± 22.0 μg/mL, respectively; $P = 0.047$). The double studied RBP-4 values were significantly similar and intraobserver agreement level was found as 0.897 (95% confidence interval, 0.856–0.926).

Spearman's rank correlation coefficient analysis revealed that maternal age and fetal birthweight were not correlated with RBP-4 values as independent variables ($r = –0.11$, $P = 0.902$; $r = 0.095$, $P = 0.811$, respec-

tively). RBP-4 values were not significantly correlated with 16–18th week FPG ($r = 0.029$, $P = 0.749$) and 50-g GCT ($r = -0.074$, $P = 0.413$) levels. RBP-4 values in the 16–18th week were not significantly different in participants with high and normal 50-g GCT (98.2 ± 17.4 vs 98.2 ± 23.0 mg/dL, respectively; $P = 0.993$). IR is expected to increase with progression of pregnancy, so a correlation analysis between the 16–18th week and 24–28th week results have not been performed.

Discussion

The present study revealed that, in the early second trimester of pregnancy, RBP-4 values do not differ between cases with or without GDM risk factors. The significantly higher FPG and 50-g GCT results, but similar RBP-4 values, in cases with GDM risk factors may be due to the impact of many factors that gradually change during pregnancy.

Yang *et al*. first demonstrated that transgenic overexpression of human RBP-4 or injection of recombinant RBP-4 in normal mice caused IR.[11] RBP-4 was suggested as the responsible factor increasing the gluconeogenic enzyme phosphoenolpyruvate carboxykinase in hepatocytes. In addition, RBP-4 also inhibits insulin receptor activity by blocking the insulin-stimulated phosphorylation of insulin receptor substrate-1 at serine in position 307. These actions of RBP-4 have been proposed to result from IR.[11,20,21] RBP-4 levels have been related with not only IR but also metabolic syndrome, type 2 diabetes mellitus, obesity and dyslipidemia.[11,12,22]

Pregnancy is a diabetogenic condition and RBP-4 values continue to rise during gestations of non-diabetic women, generally returning to normal range after delivery.[8] On the contrary, Inoue *et al*. reported that the RBP-4 values were higher in the first trimester and decrease through the second trimester and plateau thereafter in normal pregnancies.[23] Up to date, various factors have been shown to affect RBP-4 values such as iron levels,[24] renal function,[25] lipid profile,[22] transthyretin levels,[26] vitamin A levels[27] and inflammatory status (RBP-4 is a negative acute phase reactant).[28] Physiological changes such as increased iron requirement and increased glomerular filtration rate in pregnancy may also interfere with RBP-4 levels. Neither in our study nor in the previous studies has any adjustment of RBP-4 been found when the above-mentioned parameters were performed. This also makes it hard to reach a conclusion about RBP-4 levels in pregnancy.

Presence of previously mentioned risk factors for GDM are proposed to promote IR to some degree. Therefore, early GDM screening is mandatory in cases with risk factors. Considering this, in this study, RBP-4 levels were analyzed in the early second trimester rather than at routine screening weeks (24–28th weeks) in pregnant patients with GDM risk factors. According to the data from previous published work, RBP-4 values were supposed to be in the higher in risk group. However, even if the FPG and 50-g GCT values were higher in the GDM risk group, the results showed that RBP-4 levels did not differ significantly in cases with or without risk factors (95.3 ± 20.1 vs 103.1 ± 24.4 μg/mL; $P = 0.55$). Also no significant difference was found in RBP-4 values that had one or more risk factors (96.7 ± 22.6 vs 93.3 ± 16.2 μg/mL; $P = 0.468$).

In the published work, there are many studies in favor of a positive correlation between RBP-4 and IR,[11,29,30] while others support the absence of a correlation[31–34] or a negative correlation as well.[35–37] Abetew *et al*. reported that there is modest evidence for a positive association of early pregnancy elevated RBP-4 concentrations with increased GDM risk, particularly among women aged more than 35 years.[38] In our study, the number of participants aged more than 35 years were too few ($n = 9$) to perform statistical analyses. Additionally, although there was not a direct correlation between age and RBP-4 ($r = -0.011$, $P = 0.902$), RBP-4 values were significantly lower in participants aged 25 years or older compared with those of less than 25 years (93.4 ± 18.8 vs 102.2 ± 24.5 μg/mL, respectively; $P = 0.047$). On the other hand, others have shown a positive correlation between age and RBP-4 values.[15,29] The results in our study may be due to the effect of the sample size and more accurate conclusions may be reached in further large population-based studies.

Numerous studies reported higher RBP-4 values in pregnant subjects complicated with GDM.[13,39] Others proposed a negative correlation[37] or denied existence of any correlation.[40] RBP-4 values in a pregnant population who have different IR states were studied at 24–28 weeks of pregnancy with ELISA and also confirmed by western blot, resulting in no significant difference in RBP-4 values in GDM, glucose intolerance and high 50-g GCT but normal 100-g OGTT subgroups (36.0 ± 10.4 vs 35.6 ± 10.9 vs 34.6 ± 6.7 μg/mL, respectively; $P = 0.69$).[41] In spite of all technical issues, Maghbooli *et al*. suggested that RBP-4 value over 42 μg/mL determined by ELISA at 24–28 weeks of pregnancy may predict future GDM.[42] Very recently,

Abetew *et al*. reported that RBP-4 values in the 16th week of pregnancy are associated with increased GDM risk only among women particularly over 35 years of age.[38] Before a universally accepted laboratory technique is available for determining RBP-4 levels in pregnancy, all the previous data in the published work needs to be validated.

Retinol-binding protein-4 values in the published work are measured by different techniques such as ELISA, competitive enzyme immunoassay and western blot. Graham *et al*. reported that the most reliable results were available with western blot technique.[27] Howevre, ELISA has been the most frequently preferred technique to measure RBP-4 levels.[43] In the published work, the wide range of reported RBP-4 values measured by ELISA is quite remarkable. In pregnancy, the previously reported RBP-4 values ranged from 0.0169 ± 0.005 ng/mL to 41.14 ± 21.29 μg/mL.[9,38] Therefore, it is hard to form any conclusions about RBP-4 levels in pregnant subjects.

The present study was designed as a cross-sectional study but because secondary outcomes such as routine second trimester GDM screening results, route of birth and fetal birthweights were also included, the subjects who did not attend routine visits and did not give birth in the same hospital were excluded in the statistical analysis due to incomplete data. As a result, the sample size of the study was limited. Therefore, this study can be evaluated as a preliminary work for further research.

In order to clarify any existence of an association between RBP-4 levels with IR in pregnancy, adjustment for all the possible variables related to pregnancy should be performed. The controversies over the association between RBP-4 and IR may be resolved with confirmation of the previous correlations – either positive or negative – with the most reliable available laboratory technique for RBP-4 measurement currently being western blot.

Disclosure

The authors have no relevant financial or non-financial relationships to disclose.

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