THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/j.1447-0756.2012.01870.x J. Obstet. Gynaecol. Res. Vol. 38, No. 11: 1286–1293, November 2012

Sex-hormone-binding globulin early in pregnancy for the prediction of severe gestational diabetes mellitus and related complications

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Abstract

Aims: The aim of this study was to evaluate the predictive value of sex-hormone-binding globulin (SHBG) for the diagnosis of gestational diabetes mellitus (GDM), and to clarify the association between SHBG levels and GDM complications/medication requirements.

Material and Methods: Among the participants ($n = 93$) who provided blood samples between 13 and 16 weeks' gestation, 30 cases subsequently developed GDM. Complications and medical interventions were noted. The best cut-off point of SHBG and diagnostic performance were calculated.

Results: The mean age was 28.45 ± 5.0 years. SHBG levels were lower in the GDM group ($n = 30$) when compared with non-GDM ($n = 63$) cases (<0.01). Among the GDM women, SHBG was lower in the insulin therapy group ($n = 15$) compared with medical nutritional therapy alone ($n = 15$) ($P < 0.01$). A good predictive accuracy of SHBG was found for GDM requiring insulin therapy (area under the curve: 0.866, 95% confidence interval: 0.773–0.959). An SHBG threshold for 97.47 nmol/L had a sensitivity of 80.0%, specificity 84.6%, positive predictive value 50.0% and negative predictive value 95.7%. The calculated odds ratio for SHBG < 97.47 nmol/L was 12.346 (95% confidence interval: 1.786–83.33).

Conclusions: SHBG is valuable for screening women early in pregnancy for GDM risk; however, a standard assay for analyses and a threshold level of serum SHBG for a constant gestational week has to be determined.

Key words: gestational diabetes mellitus, insulin therapy, perinatal outcome, prediction of gestational diabetes, sex-hormone-binding globulin.

Introduction

Pregnancy is characterized by endocrinologic and metabolic changes to ensure energy and nutrient supply to the fetus. Placental diabetogenic hormones cause insulin resistance and hyperinsulinemia, which predispose diabetes development in pregnancy. Abnormal glucose tolerance first recognized in pregnancy is defined as gestational diabetes mellitus (GDM). The significance of gestational diabetes in pregnancy is due to adverse maternal and neonatal outcomes, including pre-eclampsia, birth trauma, macrosomia, polyhydramnios and operative delivery.[1,2]

The diagnosis and appropriate treatment of GDM can decrease maternal and fetal complications.[3,4] Therefore, 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 (ADA) and the American College of Obstetrics and Gynecologists (ACOG) recommend routine screening for GDM in pregnancy.[5,6] Screening

Received: July 22 2011.
Accepted: January 22 2012.
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all pregnant women for GDM at 26–28 weeks of gestation with a glucose challenge test followed by diagnostic testing in women who screen positive is a limitation in the treatment of GDM. This approach leaves a short period of time for interventions until delivery, like diet or medication. In addition, such an approach is complicated and costly.

Currently, early diagnostic test is performed in pregnant women with obesity, personal history of gestational diabetes, glycosuria or family history of diabetes. However, early screening of all pregnant women will help to identify GDM cases that will lead to earlier interventions and might 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.[7–9] Among these markers, sex-hormone-binding globulin (SHBG) levels in the first trimester were suggested as a valuable screening test for GDM.[10] However, the association between early pregnancy SHBG levels and fetal/maternal complications of GDM, and medical interventions are lacking in the literature. Therefore, this study was designed to evaluate the predictive value of SHBG early in the second trimester for the diagnosis of GDM and to clarify the association between SHBG levels and GDM complications and medication requirements.

Methods

This is a prospective cross-sectional study among patients who were admitted to a university clinic for routine antenatal follow up between April 2010 and March 2011. The study population consisted of the patients eligible for the study during this period. The study was approved by the research ethics committee of the university. All participants gave informed consent before enrollment to the study and all were carrying singleton gestations. The age, prepregnancy weight, gravidity, parity, and family history of diabetes were noted at admission. Participants who provided blood samples at 13–16 weeks' of gestation, completed prenatal care and delivered a live term infant after 36 weeks in our institution were included in the study ($n = 93$). The exclusion criteria were pregestational diabetes mellitus, pre-eclampsia or gestational/chronic hypertension (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg), fetal congenital anomaly, multiple pregnancies and smoking.

Maternal blood samples for SHBG were collected from the antecubital vein into a non-heparinized tube in early second trimester (between 13 and 16 weeks of gestation). Samples were immediately centrifuged, and serum was separated and frozen at –80°C until assayed for SHBG analyses. SHBG was measured from thawed serum samples with radioimmunoassay (RIA) that has intra- and interassay coefficients of variation 5.6–6.1% and 8.3–8.6%, respectively. The sensitivity of the SHBG assay was 0.2 nmol. The kits were supplied by Immunotech. At the time serum samples for SHBG were collected, maternal weights were measured. The records of systolic (SBP) and diastolic blood pressure (DBP) measured at the third trimester twice in the right arm in a relaxed sitting position were used for the analyses. The average of two measurements taken 15 min apart were used.

A glucose challenge test (50 g in all women) was performed at 24–28 weeks of gestation in all participants.[11] Screen-positive (plasma glucose ≥140 mg/dL) women further underwent a 100-g glucose tolerance test (GTT). The normal plasma glucose levels of 3-h GTT is as follows: fasting <105 mg/dL, 1 h <190 mg/dL, 2 h <165, 3 h <145 mg/dL. Screen-negative (plasma glucose <140 mg/dL in 50 g) or one abnormal plasma glucose level in 100-g GTT were considered as not having GDM. If two of the four plasma glucose levels were abnormal in 100-g GTT (≥105, 190, 165 and 145 mg/dL) then the diagnosis of GDM was made.[11] Plasma glucose was determined with the glucose hexokinase method (Cobas Integra 400 Plus).

GDM-related complications like polyhydramnios (amniotic fluid index >20 cm), macrosomia (birthweight >4500 gr) and interventions like diet or medication (insulin) were noted. Maternal weight and gestational age at birth were obtained from medical records. Birthweight of the neonates, infants with jaundice, seizures, treatment for sepsis, resuscitation at birth, or admission to the neonatal intensive care unit (NICU) were recorded.

Statistical analyses

Data analysis was performed by using SPSS for Windows, version 11.5. Whether the distributions of continuous variables were normal or not was determined by the Shapiro–Wilk test. Data are shown as mean ± SD or median (min–max), where applicable. The mean differences between groups were compared by Student's *t*-test, otherwise the Mann–Whitney *U*-test was applied for the comparisons of the median values. Nominal data were analyzed by χ^2-test or Fisher's exact test where appropriate. Degrees of association between continuous variables were

Table 1 Baseline characteristics of the GDM and non-GDM groups

Parameter	GDM ($n = 30$)	Non-GDM ($n = 63$)	P
Age (years) Mean ± SD	30.4 ± 5.9	27.5 ± 4.2	<0.01†
Gravidity Median (min–max)	2 (1–5)	1 (1–5)	NS
Parity Median (min–max)	1 (1–3)	1 (1–3)	NS
Maternal weight prepregnancy (kg) Mean ± SD	62.6 ± 6.8	62.8 ± 9.9	NS
Maternal weight at serum sampling (kg) Mean ± SD	64.5 ± 6.3	64.7 ± 9.3	NS
Maternal weight at birth (kg) Mean ± SD	72.9 ± 6.4	76.5 ± 9.0	<0.05†
Family history of diabetes (%)	66.7%	11.1%	<0.001†
SBP (mmHg) Mean ± SD	104 ± 14	97 ± 10	<0.01†
DBP (mmHg) Mean ± SD	70 ± 7	66 ± 6	<0.05†

†Statistically significant. DBP, diastolic blood pressure; GDM, gestational diabetes mellitus; NS, not significant; SBP, systolic blood pressure; SD, standard deviation.

calculated by Spearman's rank correlation analyses. The area under the curve (AUC) and 95% confidence interval (CI) for SHBG determination of GDM and insulin usage was evaluated by receiver–operator curve (ROC) analysis. The best cut-off point of SHBG and diagnostic performance, such as sensitivity, specificity, positive and negative predictive values, were also calculated. First of all, the cut-off points with 80%, 85% and 90% sensitivity were found and the specificity, positive and negative predictive values of these cut-off points were calculated. Afterwards, the cut-off points with 80%, 85% and 90% specificity were found and the sensitivity, positive and negative predictive values of these cut-off points were calculated. The optimal cut-off point was found after this evaluation. The multiple logistic regression backward method was used to determine the independent predictors that mostly affected GDM. Any variable whose univariable test had a P-value < 0.25 was accepted as a candidate for the multivariable model along with all variables of known clinical importance. Odds ratio and 95%CI for each independent variable were also calculated. A P-value less than 0.05 was considered statistically significant.

Results

Among the participants ($n = 93$), screen-negative cases (plasma glucose <140 mg/dL in 50 g) and one abnormal plasma glucose level in 100-g GTT constituted the non-GDM group ($n = 63$). The remaining 30 cases had GDM diagnosed with two abnormal plasma glucose levels in 100-g GTT (GDM group). The mean age of the women was 28.45 ± 5.0 years. The baseline characteristics of the two groups are given in Table 1. The women with GDM were found to be older than non-GDM cases and family history of GDM was more prevalent among GDM cases. SBP and DBP were statistically significantly higher in GDM cases when compared with the non-GDM group (Table 1).

Fasting plasma glucose levels at serum sampling was found to be significantly higher in GDM cases. As expected, SHBG levels were statistically significantly lower in the GDM group when compared with non-GDM cases (Table 2). The plasma glucose levels at screening (50 g) and 100-g GTT results are given in Table 2. The SHBG levels of screen-positive cases ($n = 42$) were lower than screen-negative patients ($n = 51$) (98.4 ± 3.6 nmol/L vs 99.9 ± 3.2 nmol/L, $P < 0.01$). When the association between SHBG levels and 100-g GTT results was analyzed, none of the 100-g GTT plasma glucose levels (1, 2, 3 h) were found to be associated with SHBG levels (1 h, $P = 0.09$; 2 h, $P = 0.097$; 3 h, $P = 0.391$).

All the GDM women were under medical nutritional therapy but 15 (50%) required additional insulin therapy to achieve good glycemic control. When the SHBG levels in GDM cases are analyzed regarding insulin therapy, it was found that SHBG were lower in

Table 2 Sex-hormone-binding globulin, 50-g screening and 100-g GTT results in GDM and non-GDM groups

Parameter (Mean ± SD)	GDM ($n = 30$)	Non-GDM ($n = 63$)	P
SHBG (nmol/L)	97.8 ± 2.9	99.9 ± 3.6	<0.01†
Fasting PGL at serum sampling (mg/dL)	87.0 ± 12.3	78.87 ± 6.6	<0.01†
50-g PGL (mg/dL)	170.2 ± 21.4	117 ± 26.1	<0.001†
100-g fasting PGL (mg/dL)	83.0 ± 10.2	80.8 ± 9.4	NS
100-g 1 h PGL (mg/dL)	183.6 ± 16.3	142.0 ± 24.1	<0.01†
100-g 2 h PGL(mg/dL)	158.0 ± 16.6	126.6 ± 16.5	<0.01†
100-g 3 h PGL (mg/dL)	124.7 ± 32.3	92.8 ± 30.4	<0.05†

†Statistically significant. GDM, gestational diabetes mellitus; GTT, glucose tolerance test; NS, not significant; PGL, plasma glucose levels; SD, standard deviation; SHBG, sex-hormone-binding globulin.

Table 3 The gestational and birth parameters in GDM and non-GDM groups

Parameter	GDM ($n = 30$)	Non-GDM ($n = 63$)	P
Polyhydramnios n (%)	4 (13.3)	1 (1.6)	†
Birthweight (kg) Mean ± SD	3464 ± 298	3346 ± 376	NS
Gestational age at birth (days) Mean ± SD	271 ± 5	273 ± 6	NS

†Statistical analysis not available. GDM, gestational diabetes mellitus; NS, not significant; SD, standard deviation.

the insulin therapy group ($n = 15$) compared with medical nutritional therapy alone ($n = 15$) (96.0 ± 1.4 nmol/L vs 99.7 ± 2.8 nmol/L, respectively, $P < 0.01$). During the follow up of the participants, polyhydramnios occurred in 13.3% of GDM cases and in 1.6% of non-GDM cases (Table 3). Moreover, SHBG levels of GDM cases ($n = 4$) with polyhydramnios were lower than SHBG level of the non-GDM polyhydramnios case ($n = 1$) but the number of cases was too low for statistical analyses (96.4 ± 1.8 nmol/L vs 99.4 ± 3.5 nmol/L).

The birthweights and gestational ages at birth are given in Table 3. Fetal macrosomia was not observed. None of the neonates had an Apgar score at 5 min of age < 7. None required resuscitation at birth. Neither seizures nor sepsis was observed in any of the neonates. Treatment for neonatal jaundice was performed in 12 neonates: six in each group. Three neonates were admitted to the NICU for tachypnea. The follow-up visits of all neonates were uneventful.

According to the Spearman's rank correlation analyses the baseline characteristics (age, gravidity, parity, maternal weight, SBP, DBP), 100-g GTT (1, 2, 3 h), polyhydramnios, and birthweight were not correlated with SHBG levels. However, fasting plasma glucose levels at serum sampling and 50-g screening levels were found to be negatively correlated with SHBG levels (r = –0.254, $P = 0.014$ and r = –0.382, $P = 0.000$, respectively). In addition, a positive correlation was found between gestational age at birth and SHBG (r = 0.222, $P = 0.033$).

The predictive value of the parameters on the risk for subsequent GDM development was examined by multivariable analysis using the variables that might be associated with GDM development. The results of the ROC analysis of the final model in logistic regression analysis of the statistically significant continuous variables other than SHBG are given in Table 4. The risk of development of GDM according to cut-off values is also calculated in Table 4. The predictive accuracy of SHBG early in gestation as a marker for GDM was found by ROC analysis (AUC: 0.675, 95%CI: 0.555–0.795, Fig. 1). The cut-off point 97.47 had the best sensitivity and positive predictive value in this evaluation. An SHBG threshold for 97.47 nmol/L had a sensitivity of 46.7%, specificity 84.1%, positive predictive value

Table 4 The results of the logistic regression analyses for the prediction of GDM

Independent variables	OR	Wald	*P*-value	95%CI	
				Lower limit	Upper limit
Maternal age	1.213	3.857	0.050†	1.000	1.470
Family history of GDM	17.832	11.022	<0.001†	3.255	97.692
DBP	1.148	4.867	0.027†	1.016	1.297
SHBG < 97.47	12.303	6.496	0.011†	1.786	84.773

†Statistically significant. CI, confidence interval; DBP, diastolic blood pressure; GDM, gestational diabetes mellitus; OR, odds ratio; SHBG, sex-hormone-binding globulin.

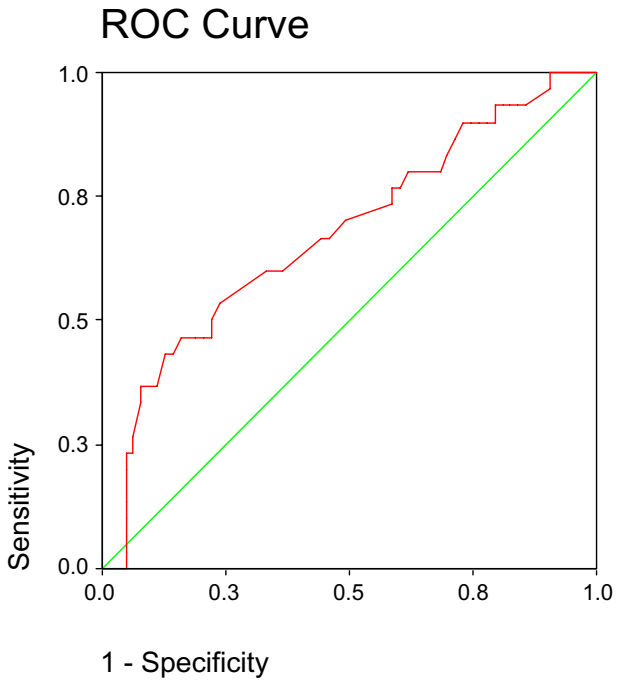

Figure 1 Receiver–operator curve (ROC) showing the predictive probabilities of early second-trimester sex-hormone-binding globulin levels for gestational diabetes mellitus. Diagonal segments are produced by ties.

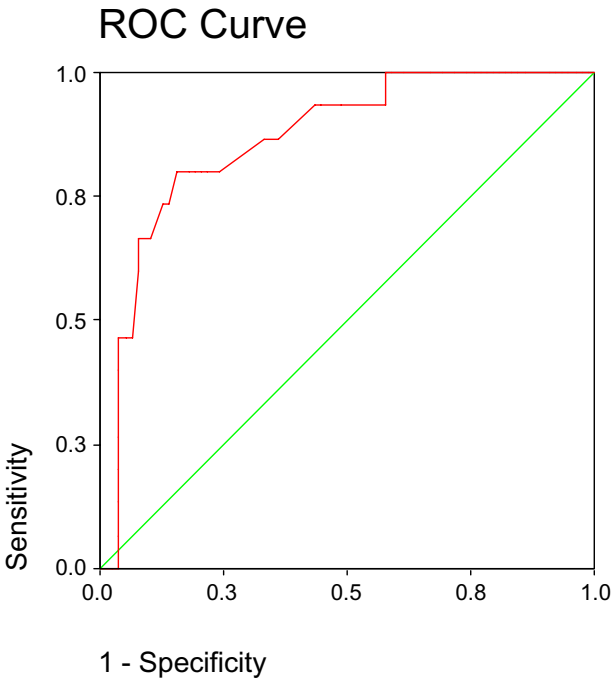

Figure 2 Receiver–operator curve (ROC) showing the predictive probabilities of early second-trimester sex-hormone-binding globulin levels for gestational diabetes mellitus requiring insulin therapy. Diagonal segments are produced by ties.

58.3% and negative predictive value 76.8%. A better predictive accuracy of SHBG was found for GDM requiring insulin therapy (AUC: 0.866, 95%CI: 0.773–0.959, Fig. 2). An SHBG threshold for 97.47 nmol/L had a sensitivity of 80.0%, specificity 84.6%, positive predictive value 50.0% and negative predictive value 95.7%.

Discussion

SHBG is important for the transport and regulation of distribution of sex steroids. Plasma SHBG is secreted in the liver under hormonal and nutritional control. In the human hepatoma cell line (HepG2), thyroid and estrogenic hormones increase SHBG. On the other hand, induced lipogenesis by monosaccharides, like glucose and fructose, decrease SHBG expression.[12] Because of the inhibitory effect of both insulin and insulin-like growth factor-1 on SHBG secretion by HepG2 cells *in vitro*, it has been proposed that SHBG levels could be a marker of insulin resistance and/or hyperinsulinism in humans.[13] Low levels of SHBG are a strong predictor of risk of type 2 diabetes mellitus in women and men.[14] The inverse association of SHBG with risk of type 2 diabetes mellitus is stronger in women than in men.[15] GDM is a

state of insulin resistance in pregnancy that seems to result from similar mechanisms in type 2 diabetes mellitus. Therefore SHBG is also an area of research in GDM but there are a limited number of studies evaluating the value of SHBG levels in GDM. In normal pregnancy, SHBG levels rise steadily until 24 weeks of gestation, remaining stable thereafter.[16,17] Probably, hyperinsulinemia and insulin resistance, which also increase progressively in normal pregnancy, may prevent further increases in SHBG.[18,19]

Previously, Stefan *et al*.[20] suggested that lipogenesis and hepatic steatosis may be determinants of circulating SHBG. The authors[20] reported that liver fat, but not visceral fat or total body fat, was an independent predictor of levels of SHBG.[20] Although body mass index and maternal lipid profile of the participants are missing in our study, maternal weight (prepregnancy, at serum sampling and at birth) was not correlated with SHBG levels. In contrast, in a cross-sectional study, second-trimester SHBG was correlated with body mass index.[21] Elevated levels of triglycerides in pregnancy might explain the potential role that lipogenesis may play in suppressing levels of SHBG and development of insulin resistance. In addition, hyperinsulinemia induced by insulin resistance in pregnancy probably causes lower levels of SHBG in higher insulin-resistant conditions, such as GDM. One of the initial studies about SHBG in GDM reported that insulinemia was similar in normal and gestational diabetic pregnant women and the authors suggested that GDM is characterized by a higher peripheral insulin resistance.[22] The lower SHBG levels in GDM cases in our study support the previous data.[22,23]

The results of the study[23] evaluating SHBG serum levels by enzyme-linked immunosorbent assay system from samples collected between 20 and 30 weeks of gestation revealed significantly lower levels of SHBG in patients with GDM than pregnant women with normal glucose tolerance, as in our study ($P < 0.01$). In addition, much lower SHBG levels were observed in GDM cases with insulin therapy.[23] This study indicates that SHBG levels at the time of routine screening and diagnosis of GDM might help to differentiate the cases that will require insulin therapy in the third trimester. However, this information does not add much to routine screening and diagnosis. In order to improve the perinatal outcomes and patient guidance, a test performed earlier in pregnancy will be more beneficial.

The first study evaluating the predictive value of first-trimester SHBG levels reported an association between SHBG levels at 10 weeks of gestation with an increased risk of the subsequent development of GDM, independent of maternal weight, age and race.[7] The authors[7] measured SHBG levels with an immunometric assay and found that women with an SHBG level of ≤175 nmol/L had a twofold increased risk of the development of GDM (odds ratio [OR]: 2.2; 95%CI 1.1–4.5). The study[10] performed to select an optimal early marker associated with the later diagnosis of GDM in a single cohort evaluated SHBG, high-sensitive C-reactive protein, and measures of fasting glucose and insulin obtained at <20 weeks. Among these three markers, first-trimester non-fasting SHBG appeared to be the optimal marker to predict subsequent GDM.[10]

Moreover, a very recent screening study performed to develop a model for the prediction of GDM from maternal characteristics and biochemical markers at 11–13 weeks' gestation showed a detection rate of 61.6% at a false positive rate of 20% by maternal characteristics (maternal age, body mass index, racial origin, previous history of GDM and macrosomic infant).[24] The authors[24] reported 74.1% detection by addition of adiponectin and SHBG. The good predictive accuracy of SHBG in early pregnancy as a marker for severe GDM was found in our study. The optimum calculated threshold of 97.47 nmol/L had a sensitivity and specificity of 80% and 84%, respectively, for GDM requiring insulin. On the basis of these results, there would appear to be potential benefit in using SHBG early in gestation for the prediction of risk of severe GDM as the calculated OR for SHBG < 97.47 was 12.346 (95%CI: 1.786–83.33). Unfortunately, due to lack of standardization of the laboratory assays used in studies and limited sample sizes, it is hard to determine a clinically useful cut-off value. In addition, most of the studies did not report the details of SHBG analyses, which makes it hard to discuss. Usually, SHBG measures are performed with antibody-based assays which are more available in standard hospital settings. However, the levels of SHBG were suggested as unreliable if performed with these assays.[25] Therefore, in our study, RIA was used to detect SHBG levels because of its great sensitivity.

Additionally, preconception SHBG levels were also strongly associated with subsequent development of GDM in women with polycystic ovary syndrome (PCOS).[26] PCOS is a common endocrinopathy with high prevalence of metabolic abnormalities, like obesity, insulin resistance and dyslipidemia.[24] The preconceptional presence of insulin resistance, like in PCOS, is amplified by the insulin-inhibiting hormones

of pregnancy. Therefore SHBG was suggested as selective screening of women at higher risk of developing GDM. The authors reported that preconception SHBG threshold of 58.5 nmol/L had a sensitivity and specificity of 81% and 82%, respectively.[26] The usefulness of preconceptional SHBG measure as a screening test for GDM in an unselected population and optimal threshold of SHBG for all women planning pregnancy might be reported in the near future after larger prospective studies.

The main adverse impacts of GDM on pregnancy are fetal macrosomia and pregnancy-induced hypertension.[27,28] In our study, although women subsequently diagnosed with GDM had significantly higher systolic and diastolic blood pressures compared with normal pregnancies, no correlation was found between early gestational age SHBG levels and blood pressures. In a previous study, second trimester maternal plasma SHBG concentrations were significantly lower in women who subsequently developed pre-eclampsia than in women with normal pregnancy outcomes.[21] On the contrary, in another study, first-trimester maternal serum SHBG concentrations were no different from controls in women who subsequently develop pre-eclampsia and pregnancy-induced hypertension.[29] Other than GDM, miscarriage was another adverse pregnancy outcome reported to occur in women with reduced levels of first-trimester SHBG levels.[29] As far as we know, there is no data reporting the association between GDM-related adverse pregnancy outcomes and early SHBG levels. In our study, although SHBG levels were much lower among polyhydramnios cases under good glycemic control, evidence is not strong to conclude polyhydramnios occurring as a complication of GDM. In addition, fetal macrosomia was not observed in our study population. The neonatal outcomes of the cases were quite favorable in this study but a limited number of cases hindered us from making a conclusion about SHBG and perinatal outcomes.

An acceptable early marker for GDM needs to be developed and SHBG seems to be the best practical option available now. Identifying women at high risk of developing GDM in a timely manner will aid to prevent the evolution of insulin resistance to GDM with dietary interventions and physical activity. Another sustained benefit will be observed in perinatal outcomes if GDM is predicted early in gestation. We infer that SHBG is valuable for screening pregnant women early in pregnancy as the opportunity for timeliness of interventions aimed at maternal glycemic control and prevention of adverse pregnancy outcomes becomes possible. But before that, a standard assay for analyses and a level of serum SHBG below which it would predict GDM at a constant gestational week will be determined.

Acknowledgments

The authors wish to thank all patients for their participation in this study, and all personnel at the Obstetrics and Gynecology Department for their enthusiastic contribution. This study had no financial support.

Disclosure

The authors declare no conflict of interest.

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