*LETTER TO THE EDITOR*

# LOWER VITAMIN D AND SEX HORMONE BINDING GLOBULIN LEVELS AND HIGHER PROGESTERONE, CORTISOL AND t-PA LEVELS IN EARLY SECOND TRIMESTER ARE ASSOCIATED WITH HIGHER RISK OF DEVELOPING GESTATIONAL DIABETES MELLITUS

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To the Editor,

Pregnancy has been associated with a diabetogenic environment, characterized by hyperinsulinemia and hyperglycemia (1). Gestational diabetes mellitus (GDM) is a disorder with variable severity with onset or first recognition of glucose intolerance during pregnancy (2). It affects approximately 1–14% of all pregnancies and its incidence is increasing steadily. GDM patients are diagnosed during the second or third trimester of pregnancy. GDM is a serious complication of the pregnancy which can elevate the risks of various maternal-fetal disorders, including premature delivery, shoulder dystocia or birth injury, macrosomia and preeclampsia (3).

Currently GDM diagnosis is made in the early third trimester, possibly exposing the infant for a significant period of time to intrauterine metabolic alterations and epigenetic programming. Identification of early biomarkers in pregnant women who later develop GDM may lead to a better understanding of GDM pathogenesis. The combination of biomarkers and risk factors into a predictive model can add to GDM early prediction, evoke effective prevention strategies and ultimately reduce GDM-related complications (4).

Deficiency of vitamin D is common throughout pregnancy and contributes to glycaemic abnormal control (5). Recent evidence suggests that, in addition to its classical roles in calcium and bone metabolism, vitamin D is involved in glucose homeostasis (6). Sex hormone binding globulin (SHBG) is a liver-based glycoprotein linked to circulating estradiol and testosterone. Insulin suppresses secretion of estradiol and testosterone, and SHBG levels are inversely linked to insulin levels and insulin resistance. Placental hormones, such as human placental lactogen, progesterone, cortisol, growth hormone and prolactin, are the major factors contributing to GDM (7). The present study was, therefore, designed in an attempt to identify the early pregnancy hormonal markers of placentation for screening of GDM.

## MATERIALS AND METHODS

*Study subjects*

Three hundred pregnant women, 176 with positive and

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124 with negative familial background of type 2 diabetes mellitus (T2DM) or GDM, were selected from different hospitals of Lahore, Pakistan, at their first antenatal visit during the early second trimester. Informed consent, clearly mentioning the purpose of the study, was signed by the patients. All of the women were screened for GDM both at early and late stages of their pregnancy. During the early care visits of gestation, participants were asked about age, history of previous diabetes, family history of type 2 diabetes mellitus, and smoking habit. Clinical parameters, including body mass index (BMI), age, systolic and diastolic blood pressure (SBP& DBP), gravida, parity and metabolic parameters such as HbA1c, FSG, were taken into account. Table I shows the average values of the clinical characteristics of the participants.

Inclusion criteria were based on positive or negative family history of GDM and T2DM, maternal age between 18 and 40, glycated hemoglobin (HbA1c) of less than 6.5% and without hypertension, renal and cardiac diseases, and current medical treatments which could affect patient hormonal concentration, lipid profile, hepatic and renal function tests.

Subjects with prior history of GDM, T2DM multiple pregnancies, ectopic pregnancy, hypertensive disorders, history of smoking/alcohol abuse, assisted reproductive technology treatment, fetal congenital irregularities and any other confounding pathologies (hyper- or hypothyroidism), polycystic ovarian syndrome, glycated haemoglobin greater than 6.5%, renal or hepatic failure, uncontrolled endocrine or any other metabolic disorder that may influence glucose regulation were excluded. Fasting glycemia was determined and women with fasting plasma glucose (FPG) > 110 mg/dl were deemed to have undiagnosed pregestational diabetes mellitus and were therefore excluded from the study.

Ethical approval for the study was obtained from the Institutional Ethics Review Board of Punjab University.

*Blood sampling*

Cases were followed-up until the development of GDM that was diagnosed if any of the glucose levels was equal to or greater than 5.1 mmol/l (fasting), 10.0 mmol/l (1 h post-load), or 8.5 mmol/l (2 h post-load). Blood was collected by venipuncture from all of the subjects after overnight fasting, at 14 to 18 weeks of pregnancy. After the determination of fasting glycemia and routine pathology testing, 5cc blood was drawn from all of the subjects and serum was separated by centrifugation. Samples were brought to room temperature before analyses. Vitamin D 25(OH)D, SHBG, progesterone, cortisol concentration and t-PA were determined using commercially available ELISA kits (DiaMetra, Italy).

*Statistical analysis*

Statistical analysis was carried out by one-way ANOVA and PROC GLM in SAS software to compare the results in comparable groups. P values ($<0.05$) were considered as statistically significant.

## RESULTS

All the subjects were followed-up until the development of GDM. The subjects who developed GDM were termed as the "GDM" group (n=58). Subjects who did not develop GDM or any other complication during follow-up were treated as the "control" group (n=100). Blood samples for both groups were collected after overnight fasting at predefined intervals; early second trimester, early third trimester (for control group) and at the onset of GDM development (for GDM group).

*Comparison between groups*

The results showed statistically significant ($P \leq 0.05$) decrease in vitamin D concentration in the GDM group compared to the control group in the early second trimester and early third trimester. Similarly, mean serum SHBG concentration for the GDM group was found to be significantly lower ($p<0.0001$) than controls at both early second and third trimesters (Table II). Only 58 of the 300 patients developed GDM, therefore Table I includes only GDM developed cases, and 100 of the 300 subjects who did not develop GDM were taken as a control group.

Statistically significant ($P \leq 0.05$) increase in progesterone level was observed in the GDM group compared to the control group. Similarly, the GDM group showed significant increase in t-PA, not only in early second trimester, but also in the early third trimester in comparison with controls. Mean cortisol concentration was significantly higher in the GDM

**Table I.** *Average (means±SEM) demographic variables of control group and GDM at early third trimester (24-28th weeks of gestation).*

| Parameters | Control group n=40 | GDM group n=50 | t- values | p-values | % difference controls *vs* GDM patients |
|---|---|---|---|---|---|
| **Age (years)** | $28.38^{a}$±0.64 | $29.12^{a}$±0.97 | -0.641 | 0.5231 | 4%↑ |
| **BMI (kg/m$^2$)** | $27.63^{b}$±0.23 | $29.43^{a}$±0.23 | -5.490 | < .0001*** | 7%↑ |
| **HbA1c (%)** | $4.97^{b}$±0.10 | $7.61^{a}$± 0.14 | -15.474 | < .0001*** | 53%↑ |
| **FSG (mg/dl)** | $88.80^{b}$± 0.55 | $119.32^{a}$±5.78 | -30.899 | < .0001*** | 33%↑ |
| **1-hr SG (mg/dl)** | $139.90^{b}$±0.76 | $182.52^{a}$±0.86 | -37.064 | < .0001*** | 30%↑ |
| **2-hr SG (mg/dl)** | $124.70^{b}$±4.18 | $161.6^{a}$±5.75 | -8.672 | < .0001*** | 29%↑ |
| **Gravida (Number)** | $1.48^{b}$ ±0.17 | $2.65^{a}$±0.10 | -54.797 | < .0001*** | 79%↑ |
| **Parity (Number)** | $0.56^{b}$ ±0.26 | $1.08^{a}$±0.48 | -19.115 | < .0001*** | 92%↑ |
| **SBP (mmHg)** | $115.72^{a}$±0.66 | $116.93^{a}$±0.57 | -1.385 | 0.1698 | 1%↑ |
| **DBP(mmHg)** | $73.29^{a}$±0.31 | $74.54^{a}$±0.30 | -2.901 | 0.0047 | 2%↑ |

*$^{a,b}$ indicate significant differences at p<0.05; Order of significance is as: a>b; BMI: Body mass index; HbA1c: glycosylated hemoglobin type A1c; FSG: Fasting serum glucose; 1-hr SG: one hour serum glucose; 2-hr SG: two hour serum glucose; *** : P<0.001*

**Table II.** *Overall comparison of hormonal profile in GDM group (n=58) and control group (n=100) at early 2nd trimester (14-18 weeks) and early 3rd trimester (24-28 weeks) of gestation.*

| Parameters | Early Second Trimester | | Early Third Trimester | | |
|---|---|---|---|---|---|
| | T2C | T2GDM | T3C | T3GDM | *P*-value |
| Vitamin D (ng/mL) | $46.72±0.94^{a}$ | $40.49±1.34^{b}$ | $31.96±0.11^{c}$ | $23.66±1.06^{d}$ | <0.0001 |
| SHBG (nmol/L) | $210.39±6.00^{a}$ | $166.84±6.75^{b}$ | $139.87±0.26^{c}$ | $105.65±3.56^{d}$ | <0.0001 |
| Progesterone(ng/mL) | $51.92±0.68^{d}$ | $61.73±1.03^{c}$ | $73.19±0.64^{b}$ | $89.52±0.93^{a}$ | <0.0001 |
| Cortisol (nmol/L) | $207.53±5.79^{d}$ | $233.79±10.05^{c}$ | $299.82±0.49^{b}$ | $340.57±10.02^{a}$ | <0.0001 |
| t-PA(ng/mL) | $5.71±0.10^{d}$ | $6.87±0.16^{c}$ | $8.33±0.16^{b}$ | $9.96±0.29^{a}$ | <0.0001 |

*$^{a,b,c,d}$ indicate significant differences at p<0.05; Order of significance is as: a>b>c>d; T2C: 2nd trimester control; T3C: 3rd trimester control; T2GDM: 2nd trimester GDM; T3GDM: 3rd trimester GDM*

**Table III.** *An overall comparison of hormonal profile in negative family history group (n=124) and positive family history group (n= 176) during early 2nd trimester (14-18 weeks) and early 3rd trimester (24-28 weeks) of gestation.*

| Parameters | Early Second Trimester | | Early Third Trimester | |
|---|---|---|---|---|
| | Negative History | Positive History | Negative History | Positive History |
| Vitamin D(ng/mL) | $43.44±0.79^{a}$ | $39.92±0.99^{b}$ | $30.32±0.47^{c}$ | $22.18±0.31^{d}$ |
| SHBG(nmol/L) | $208.86±6.01^{a}$ | $167.44±5.05^{b}$ | $114.07±2.08^{c}$ | $103.84±2.15^{c}$ |
| Progesterone(ng/mL) | $52.23±0.63^{d}$ | $59.77±0.53^{c}$ | $69.35±0.73^{b}$ | $81.11±0.74^{a}$ |
| Cortisol(nmol/L) | $206.15±4.44^{d}$ | $224.05±6.39^{c}$ | $288.35±4.39^{b}$ | $319.02±6.92^{a}$ |
| t-PA(ng/mL) | $5.32±0.13^{d}$ | $6.67±0.09^{c}$ | $7.92±0.15^{b}$ | $9.58±0.15^{a}$ |

*$^{a,b,c,d}$ indicate significant differences at p<0.05; Order of significance is as: a>b>c>d*

group when compared with controls in the early second trimester and early third trimester. All subjects showed reduced levels of vitamin D and SHBG, and elevated levels of progesterone, t-PA and cortisol in the early third trimester, but the variations were more pronounced in the GDM group as compared to the control group.

All the subjects were further categorized on the basis of positive and negative family history of GDM or T2DM. Among 300 pregnant women, the percentage of GDM pathogenesis in females with positive family history was found to be 32%, while it was 14% for those with negative family history. Hence, all the GDM subjects were further divided into two groups on the basis of positive/negative family history of GDM/T2DM. A comparison was made between these two groups to access the extent of variation. Strikingly, the changes were more pronounced in the group with positive family history. Significant increase in progesterone, cortisol and t-PA, but significant decrease in vitamin D and SHBG were observed in those with family history of GDM as compared to those without such history, both in early second and third trimesters. The results demonstrate that patients with positive family history of GDM or T2DM are more prone to develop GDM compared to those with negative family history (Table III).

## DISCUSSION

During pregnancy, women with GDM are at higher risk for maternal and fetal complications (8). Vitamin D deficiency during early pregnancy can affect the metabolism of glucose in a number of ways: pancreatic cells express hydroxylase; the active vitamin D form binds to the pancreatic cell vitamin D receptor; the human insulin gene promoter contains the vitamin D response element (9). There is also evidence of the role of vitamin D in maintaining the tolerance of glucose by affecting the secretion and sensitivity of insulin (10).

Tissue plasminogen activator (t-PA) reflects endothelial activation, but its circulating concentrations also reflect hepatic fat content, with reduced t-PA associated with weight loss. It has a remarkably strong link with insulin resistance in females with and without polycystic ovary syndrome (PCOS) (11). Significantly increased mean values of progesterone and cortisol in GDM subjects compared to the controls suggest that in pregnancy complicated with glucose intolerance there are further elevations in the levels of placental hormones which lead to insulin resistance and eventually GDM. The relationship between insulin and SHBG is well established since decreased levels of SHBG are linked with type 2 diabetes development (12). It is concluded that early 2$^{nd}$ trimester non-fasting SHBG could possibly be the best marker to asses subsequent GDM. We excluded all other factors, which could otherwise contribute to the above-mentioned alterations. Moreover, during analysis, the GDM subjects who were found to possess some other clinical complications other than GDM were also excluded. We therefore conclude that the above-mentioned alterations in pregnant women were attributed solely to GDM. Further studies are needed to assess current indicators of GDM. If levels of these studied early biomarkers can be controlled via therapeutic measures at early stages of pregnancy, the onset of said disease can be delayed or prevented in later stages of pregnancy.

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