CEOG Clinical and Experimental Obstetrics & Gynecology

Is maternal Vitamin D associated with gestational diabetes mellitus in pregnant women in Cyprus?

I. Soytac Inancli[1], E. Yayci[2], T. Atacag[2], M. Uncu[3]

[1] Department of Endocrinology and Metabolism, Near East University, Lefkoşa; [2] Department of Obstetrics and Gynecology, Near East University, Lefkoşa; [3] Department of Biochemistry, Near East University, Lefkoşa (Cyprus)

Summary

Background: There are conflicting results about the relationship between Vitamin D deficiency (VDD) and gestational diabetes mellitus (GDM). *Aim:* The aim of the study was to determine the association between 25 hydroxy vitamin D levels and GDM in Turkish Cypriot pregnant women. *Study design:* The authors conducted a prospective case-control study. *Materials and Methods:* The authors analysed serum 25OHD concentrations in 230 pregnant women between 2013-2014 in the winter period (November-April). Twenty-five hydroxy vitamin D was measured at the time of glucose tolerance testing at mid-gestation (24-26 weeks of pregnancy), in a population at increased risk for GDM. Patients were than divided in to two groups: pregnant women with GDM and pregnant women without GDM. *Results:* The mean level of 25 hydroxy vitamin D in the study group was 21.9 ±10.4 ng/ml. Pregnant women were diagnosed as having vitamin D insufficiency. The level of 25 hydroxy vitamin D in women with and without GDM were 22.3 ± 10.7 ng/dl and 20.3 ±9.3 ng/dl, respectively ($p = 0.262$). *Conclusion:* The present study showed 25OHD insufficiency in pregnant women at midgestation which was not associated with GDM.

Key words: Vitamin D deficiency; Gestational diabetes mellitus.

Introduction

Gestational diabetes mellitus (GDM) is the glucose intolerance seen in women with the onset during pregnancy. The incidence of GDM has increased in recent years partly due to the obesity epidemic and different diagnostic cut-offs taken [1, 2]. It affects 2-13% of all the pregnancies [3]. GDM has been recognized as a significant risk factor for unfavorable pregnancy outcomes. Women with GDM have a risk of developing DM in the future. In addition the children born to GDM mothers are more likely to be obese and have glucose intolerance [4, 5]. Studies are accumulating that GDM is an important contributer to the formation of Type 2 Diabetes Mellitus (DM) epidemic [6].

Vitamin D deficiency (VDD) or insufficiency have been associated with impaired glucose tolerance [7, 8]. VDD was significantly related to insulin resistance and impaired insulin secretion which was reversible with vitamin D supplementation [9, 10, 11]. Although the exact cause is unknown, a potential cause may be through the presence of vitamin D receptors in pancreatic beta cells influencing insulin secretion or through the effects of 25OHD on calcium metabolism [12, 13].

Emerging evidence suggests that Vitamin D administration can improve insulin sensitivity and glucose tolerance, but whether vitamin D supplementation can prevent GDM is unknown. Observational studies provide conflicting evidence as to whether low serum 25OHD levels are associated with GDM.

However, current evidence suggests that VDD or insufficiency may be a risk factor in the development of GDM. Some studies have shown low levels of 25OHD in patients with GDM [14,15]. Another study showed low levels of 25OHD at 16 weeks of pregnancy before the diagnosis of GDM was made [16]. It is not known whether supplementation of vitamin D can prevent GDM.

The aim of this study was to evaluate whether second trimester vitamin D was associated with GDM development during pregnancy in Turkish Cypriot women.

Materials and Methods

The present study was conducted in the Department of Obstetrics and Gynecology and Department of Endocrinology and Metabolism between January 2013 and April 2014 in the months between November and April (winter). Two hundred and thirty pregnant women were consecutively enrolled to the study. All pregnant women were residing in Cyprus. The patients attended the outpatient clinic for thier first prenatal visit and were consecutively included to the study. Exclusion criteria were patients who had a diagnosis of pregestational diabetes mellitus, chronic disease, and pregnant women with strict religious clothing.

At first visit, blood was withdrawn for glucose, urea, creatinine, ALT, AST, and TSH. Height was measured when the patient first attended and weight was measured at every visit. At the time of the study, it was a routine practice for obstetricians to prescribe prenatal multivitamins which contained calcium and vitamin D from the beginning of the second trimester. Medications and supplements taken by the pregnant women were recordered at every visit. All of the study participants were consuming prenatal mul-

Revised manuscript accepted for publication August 6, 2015

Table 1. — *Demographic characteristics of women who developed GDM and women who did not.*

Variables	GDM (n=45)	Non-GDM (n=185)	*p* value
Maternal age (years)	31.0 ± 3.9	29.5 ± 4.6	0.053
Body mass index (kg/m^2)	24.7 ± 4.6	23.2 ± 4.5	0.019
Weight gain at time of OGTT (kg)	8.4 ± 4.8	8.6 ± 4.7	0.79

Data are means ± SD. GDM: gestational diabetes mellitus;

Table 2. — *Levels of 25OHD in pregnant women with GDM and without GDM.*

Variables	GDM (n=45)	Non-GDM (n=185)	*p* value
25OHD	20.3 ± 9.3	22.3 ± 10.7	0.262
≤ 10 ng/dl	8 (17.8%)	20 (10.8%)	0.31
10.1 - 20 ng/dl	17 (37.8%)	57 (30.8%)	
20.1 - 29.9 ng/dl	12 (26.7%)	72 (38.9%)	
≥ 30 ng/dl	8 (17.8%)	36 (19.5%)	

Data are n (%).

tivitamins which contained 500 IU cholecalciferol.

Women underwent routine screening for GDM at 24-25 weeks gestation with a 12-hour fasting 75-gram oral glucose tolerance test (OGTT). Blood was withdrawn at 0, one, and two hours. Normal results were < 92 mg/dl at baseline, < 180 mg/dl at one hour, and < 153 mg/dl at two hours. The participants were then divided into two groups. The first group had a normal screening test, the second group had an abnormal glucose tolerance test and were defined has GDM (with one abnormal result after the OGTT). National Diabetes Data Group criteria were used to establish the diagnosis [17]. Blood for 25OHD was obtained at same time when 75-gram OGTT was performed (as routine practice in this clinic).

Using a standard data collection sheet, demographic characteristics, past medical history, and maternal characteristics (height and weight), were taken in the first visit. Maternal body mass index (BMI) was calculated for every participant.

The serum 25OHD concentration were determined by using a commercial electrochemiluminescence immunoassay method. Women were then followed through their pregnancy, and data were abstracted from medical records after delivery.

The authors categorized plasma 25 OH vitamin D concentrations according to the criteria Holick *et al.* published for vitamin D sufficiency (≥ 30 ng/ml), insufficiency (20-29 ng/ml), and deficiency (< 20 ng/ml) [18]; < 10 ng/ml was defined as severe deficiency of 25OHD.

Data analysis was performed by using SPSS for Windows, version 11.5. Serum glucose measurement was analyzed with the hexokinase method. Serum TSH, free T4, free T3, 25OHD, anti-thyroglobulin antibody (anti-Tg), anti-thyroid peroxidase antibody (anti-TPO) were measured by electrochemiluminescence immunoassay method. The method of measurement was carried out according to the manufacturer instructions. Informed consent was obtained from all individual participants included in the study.The study was approved by the Near East University Institutional Review Board.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statistical analysis

Because the distribution of 25OHD was approximately normally distributed, the authors examined the difference in mean concentrations between cases and controls. Whether the distributions of continuous variables were normally distributed or not was determined by Kolmogorov Smirnov test. The mean differences among groups were compared by using Student's *t*-test and Mann Whitney U test.

Results

The incidence of GDM was 19.6% (45 for GDM and 185 for non-GDM). The maternal age of both groups were not significantly different. Women who developed GDM had a higher BMI (Table 1). Pre-pregnancy BMI of 230 pregnant women was 23.5 ± 4.6 kg/m^2. The BMI of the pregnant women with and without GDM was 24.7 ± 4.6 kg/m^2 and 23.2 ± 4.5 kg/m^2, respectively (p = 0.019). There was no difference between both groups according to the pre-pregnancy and the weight gain at six months (8.4 ± 4.8 *vs.* 8.6 ± 4.7, p = 0.791).

Levels of 25OHD are shown in Table 2. Mean level of 25OHD in the study group was 21.9 ±10.4 ng/ml. The study population had 25OHD insufficiency. Severe VDD was 12.2% and 44.4 of all the participants had VDD (25OHD < 20 ng/ml) and only 19.1% of the participants had a sufficient vitamin D level. Mean level of 25OHD in pregnant women with and without GDM was 20.3 ± 9.3 ng/dl and 22.3 ± 10.7 ng/dl, respectively (p = 0.262). Although not statistically significant 25OHD deficiency (25OHD < 20 ng/ml) in pregnant women with GDM and without GDM was 55.5% and 41.6%, respectively (p = 0.19).

Although not statistically significant, 17.8% of the pregnant with GDM and 10.8% of the control group had 25OHD levels below 10 ng/dl - consistent with a diagnosis of sever 25OHD insufficiency.

25OHD insufficiency was 26.7% and 38.9% in pregnant women with and without GDM, respectively (p = 0.21). Pregnant women with sufficient levels of 25OHD were 17.8% in GDM and 19.5% in non-GDM pregnant women. Although not statistically significant in patients with 25OHD levels < 10 ng/dl, the risk of GDM was 1.784 (95% CI: 0.730 - 4.361, p = 0.2), and the risk in patients with 25OHD levels < 20 ng/dl was 1.753 (95% CI : 0.909 - 3.981, p = 0.09).

Discussion

It is not clear weather low levels of 25OHD has a causative role in the formation of GDM. In the present study, GDM was not associated with maternal plasma

25OHD. The present results were in concordance with the results of Whitelaw *et al.* who found no association between 25OHD levels and GDM [19]. The mean level of 25OHD was 9.3 ng/ml and GDM was diagnosed in 9.3% of the pregnant women. Park *et al.* found no association between the risk of GDM, insulin resistance, and impaired β cell function in Korean women [20]. Although Farrant *et al.* could not find an association between 25OHD levels and GDM in women in India, they found an inverse association between 25OHD levels and 30-minute glucose concentrations after a glucose load [21]. Makgoba *et al.* found no association between first trimester 25OHD and subsequent development of GDM, but found a inverse correlation between 25OHD levels and glucose measurements after a two-hour fasting glucose tolerance test [22]). Among 29% of the pregnant women pregnant women with 25OHD levels of < 15 nmol/L, the prevalence of GDM was significantly higher compared with women with 25OHD levels of ≥ 35 nmol/L. Burris *et al.* suggested that low levels of plasma 25OHD had an increased risk for the development of GDM and found an inverse association between glucose levels after one-hour 50-gram challenge test and low 25OHD levels [23].

Maghboli *et al.* demonstrated that maternal serum levels of 25OHD at 24-28 weeks of pregnancy were significantly lower in women with GDM compared with controls [14]. Clifton-Bligh *et al.* demonstrated an inverse association between maternal serum 25OHD and fasting blood glucose but could not demonstrate a statistically significant association between mid gestation 25OHD and GDM [24]. However Lacroix *et al.* demonstrated that low levels of 25OHD at first trimester was an independent risk factor for developing GDM [25].

The present gestational diabetic pregnant women were higher than expected. This may be due to the test and diagnostic cut-offs the authors used to define GDM.

The present study group had 25OHD insufficiency Although under vitamin D replacement treatment, the amount of pregnant women with severe 25OHD deficiency was higher than expected. Although this was not associated with GDM, the amount of vitamin D given was not sufficient. In previous studies there was controversy about the association of VDD in pregnancy and adverse pregnancy outcomes. 25OHD deficiency in pregnancy is likely to cause pregnancy outcomes such as preeclampsia, gestational diabetes mellitus, preterm birth, and small-for-gestational age [26]. So the amount of vitamin D that should be given to a pregnant women must be identified by prospective studies.

Insulin resistance and β cell function may play an important role in the development of GDM. However Park *et al.* could not find an association between insulin resistance and 25OHD levels [20].

Overweight and obesity are known risk factors that cause diabetes mellitus. Obesity is associated with both GDM [27] and low 25OHD levels [28]. In concordance with the literature, pregnant women with GDM had a slightly higher BMI score than patients without BMI which may be due to pregnancy induced insulin resistance and impaired insulin secretion [16]. However the weight change at six months was not different in both groups.

In conclusion, this was the first study performed in Cyprus. Turkish Cypriot pregnant women have vitamin D insufficiency with a mean level of 25OHD level of 21.9 ± 10.4 ng/ml in the second trimester which is not associated with increased GDM prevalence.

References

[1] Ferrara A., Kahn H.S., Quesenberry C.P., Riley C., Hedderson M.M.: "An increase in the incidence of gestational diabetes mellitus: Northern California, 1991-2000". *Obstet. Gynecol.*, 2004, *103*, 526.

[2] Ryan E.A.: "Diagnosing gestational diabetes". *Diabetologia*, 2011, *54*, 480.

[3] Hunt K.J., Schuller K.L.: "The increasing prevalence of diabetes in pregnancy". *Obstet. Gynecol. Clin. North Am.*, 2007, *34*, 173.

[4] Silverman B.L., Metzger B.E., Cho N.H., Loeb C.A.: "Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism". Diabetes Care, 1995, *18*, 611.

[5] Petitt D.J., Bennett P.H., Knowler W.C., Baird H.R., Aleck K.A.: "Gestational diabetes mellitus and impaired glucose tolerance during pregnancy. Long-term effects on obesity and glucose tolerance in the offspring". *Diabetes*, 1985, *34*, 119.

[6] Jelsma J.G., van Poppel M.N., Galjaard S., Desoye G., Corcoy R., Devlieger R., *et al.*: "DALI: Vitamin D and lifestyle intervention for gestational diabetes mellitus (GDM) prevention: an European multicentre, randomised trial - study protocol". *BMC Pregnancy Childbirth*, 2013, *13*, 142.

[7] Forouhi N.G., Luan J., Cooper A., Boucher B.J., Wareham N.J.: "Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000". *Diabetes*, 2008, *57*, 2619.

[8] Forouhi N.G., Ye Z., Rickard A.P., Khaw K.T., Luben R., Langenberg C., Wareham N.J.: "Circulating 25-hydroxyvitamin D concentration and the risk of type 2 diabetes: results from the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort and updated meta-analysis of prospective studies". *Diabetologia*, 2012, *55*, 2173.

[9] Cade C., Norman A.W.: "Rapid normalization/stimulation by 1,25-dihydroxyvitamin D3 of insulin secretion and glucose tolerance in the vitamin D-deficient rat". *Endocrinology*, 1987, *120*, 1490.

[10] Chertow B.S., Sivitz W.I., Baranetsky N.G., Clark S.A., Waite A., Deluca H.F.: "Cellular mechanisms of insulin release: the effects of vitamin D deficiency and repletion on rat insulin secretion". *Endocrinology*,1983, *113*, 1511.

[11] Norman A.W., Frankel J.B., Heldt A.M., Grodsky G.M.: "Vitamin D deficiency inhibits pancreatic secretion of insulin". *Science*, 1980, *209*, 823.

[12] Tai K., Need A.G., Horowitz M., Chapman I.M.: "Vitamin D, glucose, insulin, and insulin sensitivity". Nutrition, 2008, *24*, 279.

[13] Palomer X., Gonzalez-Clemente J.M., Blanco-Vaca F., Mauricio D.: "Role of vitamin D in the pathogenesis of type 2 diabetes mellitus". *Diabetes Obes. Metab.*, 2008, *10*, 185.

[14] Maghbooli Z., Hossein-Nezhad A., Karimi F., Shafaei A.R., Larijani B.: "Correlation between vitamin D3 deficiency and insulin resistance in pregnancy". *Diabetes Metab. Res. Rev.*, 2008, *24*, 27.

[15] Clifton-Bligh R.J., McElduff P., McElduff A.: "Maternal vitamin D deficiency, ethnicity and gestational diabetes". *Diabet. Med.*, 2008, *25*, 678.

[16] Zhang C., Qiu C., Hu F.B., David R.M., van Dam R.M., Bralley A., Williams M.A.: "Maternal plasma 25-hydroxyvitamin D concentra-

tions and the risk for gestational diabetes mellitus". *PLoS One*, 2008, *3*, e3753.
[17] Hadar E., Hod M.: "Establishing consensus criteria for the diagnosis of diabetes in pregnancy following the HAPO study". *Ann. N. Y. Acad. Sci.*, 2010, *1205*, 88.
[18] Holick M.F.: "Vitamin D deficiency". *N. Engl. J. Med.*, 2007, *357*, 266.
[19] Whitelaw D.C., Scally A.J., Tuffnell D.J., Davies T.J., Fraser W.D., Bhopal R.S., *et al.*: "Associations of circulating calcium and 25-hydroxyvitamin D with glucose metabolism in pregnancy: a cross-sectional study in European and South Asian women". *J. Clin. Endocrinol. Metab.*, 2014, *99*, 938.
[20] Park S., Yoon H.K., Ryu H.M., Han Y.J., Lee S.W., Park B.K., *et al.*: "Maternal vitamin d deficiency in early pregnancy is not associated with gestational diabetes mellitus development or pregnancy outcomes in korean pregnant women in a prospective study". *J. Nutr. Sci. Vitaminol. (Tokyo)*, 2014, *60*, 269.
[21] Farrant H.J., Krishnaveni G.V., Hill J.C., Boucher B.J., Fisher D.J., Noonan K., *et al.*: "Vitamin D insufficiency is common in Indian mothers but is not associated with gestational diabetes or variation in newborn size". *Eur. J. Clin. Nutr.*, 2009, *63*, 646.
[22] Makgoba M., Nelson S.M., Savvidou M., Messow C.M., Nicolaides K., Sattar N.: "First-trimester circulating 25-hydroxyvitamin D levels and development of gestational diabetes mellitus". *Diabetes Care*, 2011, *34*, 109.
[23] Burris H.H., Rifas-Shiman S.L., Kleinman K., Litonjua A.A., Huh S.Y., Rich-Edwards J.W., *et al.*: "Vitamin D deficiency in pregnancy and gestational diabetes mellitus". *Am. J. Obstet. Gynecol.*, 2012, *207*, 182.e1.
[24] Clifton-Bligh R.J., McElduff P., McElduff A.: "Maternal vitamin D deficiency, ethnicity and gestational diabetes". *Diabet. Med.*, 2008, *25*, 678.
[25] Lacroix M., Battista M.C., Doyon M., Houde G., Ménard J., Ardilouze J.L., *et al.*: "Lower vitamin D levels at first trimester are associated with higher risk of developing gestational diabetes mellitus". *Acta Diabetol.*, 2014, *51*, 609.
[26] Wei S.Q.: "Vitamin D and pregnancy outcomes". *Curr. Opin. Obstet. Gynecol.*, 2014, *26*, 438.
[27] Chu S.Y., Callaghan W.M., Kim S.Y., Schmid C.H., Lau J., England L.J., Dietz P.M.: "Maternal obesity and risk of gestational diabetes mellitus". *Diabetes Care*, 2007, *30*, 2070.
[28] Parikh S.J., Edelman M., Uwaifo G.I., Freedman R.J., Semega-Janneh M., Reynolds J., Yanovski J.A.: "The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults". *J. Clin. Endocrinol. Metab.*, 2004, *89*, 1196.

Address reprint requests to:
S. SOYTAC INANCLI, M.D.
Hakki Boratas Sok.
Kemal Sayin Apt.
No: 8 Daire 3, Girne
KKTC Mersin 10 (Turkey)
e-mail: inanclis@yahoo.com