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Relationship between osteocalcin levels and gestational diabetes mellitus

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Abstract

Osteocalcin is an osteoblast-derived protein mainly acting on bone formation. There is growing evidence that osteocalcin has an important role in glucose metabolism. It was not fully elucidated in gestational diabetes mellitus. The aim of our study was to investigate osteocalcin levels in gestational diabetes mellitus and its subgroups. We performed a case–control cross sectional study and evaluated all the demographic and anthropometric parameters of 80 pregnant women whose age and body mass indices similar, half of them having normal glucose tolerance (NGT) and the other half having gestational diabetes mellitus (GDM). We compared osteocalcin, calcium metabolism parameters, glucose, lipid levels, insulin resistance parameters, and investigated correlations of all parameters in NGT and GDM. We also analyzed GDM subgroups, which were classified according to age, parity, body mass index (BMI), and vitamin D levels. Osteocalcin levels were increased in patients with GDM and in older, multiparous, with low vitamin D or high body mass index GDM subgroups but these increases were not statistically significant (P>0.05). There was a positive correlation between osteocalcin and C peptide in GDM group and also in GDM subgroups with age≥30, multiparous, vitamin D≥20 ng/ml or BMI≥30 kg/m^2 (r=0.424, P=0.006; r=0.460, P=0.011; r=0.408, P=0.017; r=0.520, P=0.013; r=0.603, P=0.002 respectively). We also found a negative correlation between Osteocalcin and HDL-cholesterol in GDM and in multiparous, vitamin D≥20 ng/ml or BMI<30 kg/m^2 GDM subgroups (r=-0.334, P=0.035; r=-0.352, P=0.041; r=-0.430, P=0.041; r=-0.442, P=0.031 respectively). Our results suggest that osteocalcin may play a role in gestational diabetes mellitus especially if pregnant women are older, multiparous, and obese.

Keywords: Gestation, diabetes mellitus, osteocalcin

Introduction

Gestational diabetes mellitus (GDM) defined as glucose intolerance detected during pregnancy is a worldwide growing complication of pregnancy. Many factors have been thought to be associated such as obesity before and during pregnancy, advanced maternal age, previous history of GDM but its etiology is not fully understood.

Evidence about the reciprocal interaction between bone and energy metabolism is increasing in recent years [1,2]. Osteocalcin (OC) is an osteoblast derived protein locally acting on bone formation. Some studies showed that OC may have a role in regulation of glucose and fat metabolism [3,4]. OC level reported to be decreased in patients with type 2 diabetes mellitus (T2DM) [5] and type 1 diabetes mellitus (T1DM) [6]. In contrast to T1DM and T2DM, increased OC level was found in GDM [7] by Winhofer et al, but Saucedo et al concluded that GDM was not associated with OC [8]. Due to very few inconclusive studies about OC levels in GDM [7-12], we designed this study in Turkish GDM patients in order to investigate role of OC and its associations with glucose metabolism.

Material and Methods

TA total of 80 pregnant women, half of them with normal glucose tolerance (NGT) and the other half with gestational diabetes mellitus (GDM) were recruited from outpatient Clinic of Gynecology, Training and Research Hospital from May 2010 to August 2010. Ethical committee of Ankara Training and Research Hospital gave

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approval for the study with 2781 number. All the subjects gave written informed consent. The diagnosis of gestational diabetes was determined by the guidelines proposed by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [13], with a 75-gr 2-hour oral glucose test at 24-28 gestational weeks. GDM was diagnosed if the fasting blood glucose (FBG) was ≥92 mg/dL or the 1-hour value was ≥180 md/dL or the 2-hour value was ≥153 mg/dL. Before starting diet or insulin therapy, blood was withdrawn after 12 hours of overnight fasting for other laboratory analyses, and post-prandial blood glucose (PPBG) was determined at 1st hour of breakfast.

Pregnant women with history of pregestational diabetes mellitus, fetal abnormalities, chronic renal or liver disease, thyroid and bone disease and history of consumption of calcium (Ca) supplements or vitamin D (Vit D) during their pregnancy were excluded from the study.

We compared all parameters in NGT, GDM groups and in GDM subgroups which were classified as ages below 30 and above, nulliparous and multiparous, 25 hydroxy vitamin D (25OHD) levels below 20 ng/mL and above or body mass indices (BMI) below 30 kg/m^2 and above. We also performed correlation analysis in all these groups and subgroups.

Laboratory methods

FBG, PPBG, serum total cholesterol (TC), triglyceride (TG), HDL cholesterol (HDL-C), Ca, phosphorus (P), magnesium (Mg) were measured by spectrophotometric method in Olympus 2700 autoanalyzer (Mishima Olympus Co. Ltd- Olympus Corporation–Japan) using Olympus System Reagent kits. LDL-cholesterol (LDL-C) was calculated by the Friedewald Formula (LDL: Total cholesterol- (HDL+TG/5). Hemoglobin A1c (HbA1c) was determined by Tosoh G7 device by HPLC method. Fasting insulin (FI), C-peptide (Cpep) were calculated with DRG kits in the same device. PTH were studied by chemiluminescence method in Advia Centaur XP (Siemens Diagnostic-USA) device with its kits. 25OHD was calculated by Tandem Gold liquid chromatography with mass spectrophotometer (LC/MS/MS- Zivac Technologies-Turkey) device. We determined the levels of OC by DiaSource kits DSX automatized ELISA system device (Dynex Technologies Inc USA). An indirect measure of insulin resistance was calculated from the fasting plasma insulin (μu/ml) x fasting plasma glucose (mmol/l)/22.5 formula as homeostasis model assessment-insulin resistance (HOMA-IR). Corrected Ca was determined by the formula (Corrected Ca: Serum Ca+0.8(4-Serum albumin). Ca levels mentioned in the text were corrected Ca levels calculated by the formula.

Statistical analysis

Calculations were performed using SPSS version 11,5 (Customer ID 30000105 930). Descriptive statistics were expressed as mean ± standard deviation, median (min–max) for normally and abnormally distributed variables respectively and whereas observation number or % were used to express qualitative variables. To comparisons between groups Student-t test for parametric values, Mann Whitney-U test for non-parametric values, and Pearson-Chi square test was used for categorical variables. Spearman correlation coefficients were used for correlation analysis. P value <0.05 was considered as statistically significant.

Results

A total of 80 patients, composed of 2 groups, NGT (n:40) who had normal glucose metabolism and GDM(n:40) who had gestational diabetes were recruited for the study. Demographic and laboratory

Table 1. TCharacteristics of all pregnant women

	NGT n:40	GDM n:40		NGT n:40	GDM n:40
Age (year)	29.57±3.93	29.825±4.03	Triglyceride (mg/dL)	181.85±49.15	232.50±70.40b
Number of Pregnancy	2(1-5)	3(1-7)	T.Cholesterol (mg/dL)	220.60±34.59	234.85±46.89
BMI (kg/m2)	28.70±4.12	29.55±3.63	Trigliseride (mg/dL)	181.85±49.15	232.50±70.40***
FBG (mg/dL	78.97±6.20	92.2±17.73c	HDL-Cholesterol (mg/dL)	62.52±10.50	60.80±9.59
PPBG (mg/dL)	114.40±22.13	159.65±38.34c	HDL-Cholesterol (((mg/dL)	121.32±29.99	128.40±44.33
HemoglobinA1c (%)	5.02±0.29	5.63±0.51[c]	Trigliseride (mg/dL)	9.46±0.41	9.40±0.47
Insulin (μu/mL)	12.07±4.35	14.27±5.37a	LDL-Cholesterol (mg/dL)	3.27±0.53	3.53±0.51
C-Peptide (ng/mL)	0.91±0.57	0.99±0.53a	Trigliseride (mg/dL)	0.77±0.07	0.66±0.09
HOMA-IR	2.37± 0.97	3.27± 1.42**	Calcium (mg/dL)	35.51±21.62	37.39±17.74
			Phosphorus (mg/dL)	32.98±18.33	29.06±14.20

FBG; Fasting blood glucose, PPG: Post prandial blood glucose, HOMA-IR: Homeostasis model assessment- insulin resistance index, PTH: Parathyroid hormone, 25 OHD: 25 hydroxy vitamin D. Data are presented as mean ± SD except number of pregnancies which were presented as median (min-max). [a] P<0.05, [b] P<0.01, [c] P<0.001.

parameters of the groups and their comparisons are shown in Table 1. FBG, PPBG, HbA1c, FI, Cpep, HOMA-IR, and TG levels of GDM were significantly higher than NGT group. OC levels were non-significantly higher in GDM (1.65±1.60 vs.1.58±1.22 ng/ml, P>0.05).

In subgroup analysis, OC levels of GDM subgroups having age≥30 or multiparous or VitD≥20 ng/ml or BMI≥30 kg/m^2 were found to be higher than the opposing subgroups. The difference was not statistically significant (P>0.05). In multiparous GDM subgroup BMI (p=0.047), FI (p=0.013) and HOMA-IR (p=0.025) were statistically higher than nulliparous, and in BMI≥30 GDM subgroup Mg levels (p=0.021) were lower than those in the low BMI subgroup (Table 2).

Table 2. Characteristics of the gestational diabetes mellitus subgroups

	Age<30 n=10 %25	Age≥30 n=30 %75	Nulliparous n=6 %15	Multiparous n=34 %85	VitD<20 n=17 %42	VitD≥20 n=23 %57	BMI<30 n=24 %60	BMI≥30 n=16 %40
Age (year)	23.90±2.28	31.82±2.02[b]	30.33±3.61	29.73±4.14	29.00±4.44	30.43±3.67	29.08±3.79	30.93±4.25
Number of Preg	2(1-4)	3(1-7)	1(1-1)	3(1-7)[c]	3(1-5)	2(1-5)	2(1-4)	3(1-7)[b]
BMI (kg/m2)	27.16±4.37	30.34±3.03	26.89±2.90	30.02±3.58[a]	28.93±3.18	30.09±3.94	27.15±2.47	33.14±1.42[a]
FBG (mg/dL)	87.60±16.28	93.73±18.18	93.00±16.86	92.05±18.11	93.47±18.74	91.26±17.25	89.62±19.18	96.06±5.05
PPBG (mg/dL)	161.20±36.19	159.13±39.62	166.5±30.36	158.44±39.85	161.23±44.51	158.47±34.08	160.83±43.92	157.87±29.30
HbA1c(%)	5.58±0.77	5.64±0.41	5.61±0.28	5.63±0.55	5.62±0.63	5.61±0.41	5.57±0.57	5.71±0.42
Insulin (µu/mL)	14.16±4.38	14.31±5.73	13.41±3.77	19.16±3.77[a]	13.85±5.58	14.58±5.30	14.20±5.89	14.39±4.65
Cpep (ng/mL)	0.81±0.30	1.05±0.58	1.05±0.46	0.98±0.55	0.99±0.49	0.99±0.56	0.84±0.47	1.22±0.55
HOMA-IR	3.02±0.94	3.35±1.55	3.46±1.54	4.06±1.31[a]	3.15±1.18	3.36±1.59	3.18±1.53	3.40±1.27
PTH (pg/mL)	27.81±11.62	37.91±18.84	33.13±6.74	35.78±19.08	41.63±19.34[a]	30.77±15.23	35.52±18.94	36.18±16.39
25OHD (ng/mL)	33.21±18.75	32.91±18.52	42.28±14.83	31.34±18.58	15.10±3.74	46.16±12.59[c]	38.23±16.43	28.61±20.09
OC (ng/mL)	1.45±1.68	1.62±1.06	1.47±1.51	2.22±2.08	1.90±1.76	1.15±1.27	1.39±1.67	1.87±1.51

Number of Preg: Number of pregnancies, BMI: Body mass index, FBG: Fasting blood glucose, PPBG: Post prandial blood glucose, HbA1c: Hemoglobin A1c, Cpep: C-peptide, HOMA-IR: Homeostasis model assessment- insulin resistance index, PTH: Parathyroid hormone, 25 OHD: 25 hydroxy vitamin D, OC: Osteocalcin. Data are presented as mean ± SD except number of pregnancies which were presented as median (min-max). [a] P<0.05, [b] P<0.01, [c] P<0.001.

Table 3. Significant correlations between osteocalcin and parameters in GDM and its subgroups

	GDM	Age<30	Age≥30	Multiparous	VitD<20	VitD≥20	BMI≥30
C-Peptide	r=0.424[b]		r=0.460[a]	r=0.408[a]		r=0.520[a]	r=0.603[b]
BMI		r=0.736[a]					
HDL-C	r=-0.334[a]			r=-0.352[a]		r=-0.430[a]	r=-0.442[a]
Calcium					r=0.505[a]		
Magnesium	r=0.369[a]						

r: Pearson correlation coefficient, [a] P<0.05, [b] P<0.01.

There was a positive correlation between OC and C pep in GDM and GDM subgroups having age≥30, multiparous, VitD≥20 ng/ml, BMI≥30 kg/m^2; and a negative correlation between OC and HDL-C in GDM and GDM subgroups having multiparous, VitD≥20 ng/ml, BMI <30 kg/m^2. In addition, a positive correlation between OC and Mg was also found in GDM (Table 3).

Discussion

OC is known that it is an osteoblast-derived protein locally acting on bone formation. However, in the view of recent experimental studies, existence of a positive loop between OC and insulin is suggested, by which directly stimulates proliferation of β cells and insulin secretion, whereas the release of OC is enhanced by insulin [14-18]. In addition to bone formation and insulin secretion-enhancing effect, OC also increases peripheral insulin sensitivity through cytokines such as adiponectin [3,4,19]. In OC lacking mice, it was shown that β cells proliferation decreased, insulin resistance and visceral fat were increased [20]. In another animal study, mice lacking either one copy of the insulin receptor gene in osteoblasts, or the OC gene, high fat diet (HFD) induced a more severe form of insulin resistance than wild-type mice did [21], while selective overexpression of the insulin receptor in osteoblasts protected from HFD-induced insulin resistance. OC was reported to be decreased in patients with DM, and conversely treatment that improve glycemic control are associated with an increase in OC serum concentrations in those patients [5,6]. OC levels lessen in early pregnancy, start to rise in the third trimester until delivery, and peak during lactation (22). Papastefanou et al. demonstrated that total OC serum levels are higher during the first trimester of pregnancy in women subsequently developing GDM when compared to controls [23].Winhofer et al. found that OC was increased in GDM, which serves a model to study for the development of insulin resistance and T2DM [7], but Saucedo et al. concluded that GDM was not associated with OC [8]. In our study, HOMA-IR, FI and C-pep levels of the GDM group were significantly high, but although statistically not significant, the OC levels of the women with GDM were higher than those of NGT pregnant. Although Winhofer et al. also found positive correlation between OC and glucose, insulin, Cpep levels in his patients with GDM [7], we only observed a positive correlation between OC and Cpep in GDM group. These results made us think that OC levels may increase to compensate adverse conditions of insulin resistance by trying to increase insulin secretion from pancreatic β cells. Our study was performed only in second trimester and during the summer season; it did not cover whole pregnancy and lactation unlike those studies. Since OC and other bone turnover markers may have a seasonal variation [24] and bone turnover is a time spreading processes, long-term and recurrent measurements of OC can better demonstrate its value in GDM.

OC has been reported to be negatively correlated with BMI, VitD, FBG, HbA1c, HOMA-IR, hyperlipidemia and increased with glycemic control [25-28]. Weight loss, exercise and bariatric surgery have been found to increase OC levels [29,30]. In this study, OC levels were high but non-significant in GDM subgroups having older, multiparous, VitD deficiency or obese. There was a positive correlation between OC and Cpep in GDM subgroups having older, multiparous, high VitD or obese. We also found a negative correlation between OC and HDL-C in multiparous or with high VitD or low BMI GDM subgroups as in GDM group. Our multiparous GDM subgroup had higher BMI levels than nulliparous ones. Multiparity may be a preparatory factor for obesity and metabolic disorders, one of which is low HDL-C. We think again that in GDM women OC levels may increase to compensate adverse metabolic conditions of insulin resistance such as dyslipidemia, but we only demonstrate this effort by the correlation of only between OC and Cpep, and HDL-C. The reason why we cannot find the same correlation in all our groups may lie in the low number of our subgroups

OC is a bone matrix protein that regulates hydroxyapatite size and shape through its Vitamin K dependent carboxylated form. Up to 50% of Ost is incompletely carboxylated (undercarboxylated OC) in humans [31]. The undercarboxylated form of OC is reported to be active in glucose metabolism in mice [17]. Total serum OC concentrations in humans are inversely associated with measures of glucose metabolism. However, human data are inconclusive with undercarboxylated OC because most studies do not account for the influence of Vitamin K on the proportion of undercarboxylated OC [32,33]. In our study it can be thought that the missing findings about the OC may stem from these differences.

Magnesium plays an important role in carbohydrate metabolism and may affect the release and activity of hormones that help controlling blood glucose level by tyrosine kinase [34]. In addition, insulin partially regulates intracellular magnesium accumulation, and Mg deficiency during pregnancy impairs insulin sensitivity [34]. Mg levels were non-significantly low in our patients with GDM but in obese GDM subgroup, Mg levels significantly lower than those nonobese subgroup. Positive correlation between OC and Mg, also C pep in GDM was thought that OC might increase in order to improve the insulin sensitivity.

Our study had some limitations as like the moderate sample size in all groups; OC was evaluated on one point in summer season; undercarboxylated OC couldn't be measured and the findings are limited to Turkish groups that results may not be applicable to other nationalities.

Conclusion

In conclusion we decided that OC might play a role in Turkish GDM patients and it increases in order to cope with beta cell dysfunction and insulin resistance, which are important in GDM etiopathogenesis. Although, we could not demonstrate statistically significant increase in OC levels in our GDM patients and in all subgroups, probably due to our relatively small sizes of groups and subgroups, our results suggest that the conditions leading to insulin resistance such as older age, multiparity or high BMI may related to higher level of OC. Carefully designed studies measuring carboxylated and undercarboxylated OC throughout

the pregnancy and postpartum period are required to define the role of OC in the regulation of glucose metabolism.

Competing interests
The authors declare that they have no competing interests.

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Ethical approval
Approval for the study was granted by ethical commitee of Ankara Education and Research Hospital with 16.12.2009 date and 2781 number.

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