Osteocalcin and cross laps Status among Women with Gestational Diabetes Mellitus during Pregnancy

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Abstract

Background: The aim of the study was to compare the bone turnover in healthy pregnant women and patients with Gestational Diabetes Mellitus (GDM).
Methods: A cross-sectional study was conducted on 695 pregnant women referred to five teaching hospital clinics. Universal screening was performed with Glucose Challenge Test (GCT)-50 g. Those with plasma glucose levels ≥ 7.2 mmol/L were diagnosed as GDM if they had an impaired GTT-100 g based on Carpenter and Coustan criteria. The levels of insulin and C-peptide were measured during OGTT-100 g test. The homeostasis model assessment index (HOMA) equation was used as the insulin resistance index. The concentrations of Osteocalcin, cross laps, and calcium were also measured.
Results: There was a significant difference in Osteocalcin and Crosslaps levels between GDM and normal groups. Concerning bone markers and insulin resistance, after adjusting for BMI and age, in regression model the HOMA index revealed significant relation with serum levels of Crosslaps (P= 0.03, β = 0.16) .
Conclusion: Our study showed a higher bone turnover in GDM patients during pregnancy. Specially increased bone resorption which was independently correlated with GDM may propose common defective pathways may contribute to GDM and bone loss pathogenesis.

Keywords: Gestational Diabetes Mellitus, Bone Turnover, Osteocalcin, Crosslaps

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Introduction

The effects of pregnancy on bone condition have been studied in several researches and an increase in bone turnover has been reported during pregnancy [1-3]. During pregnancy, minerals are actively transported across the placenta to the fetal circulation against concentration gradient, and the fetus is totally dependent on maternal resources to acquire minerals [4]. Bone biopsy showed that there is a two-phase bone response during pregnancy with an early phase of bone resorption (12–14 weeks) and a later phase of bone formation (38–40 weeks) [5].

On the other hand, pregnancy is known as a diabetogenic condition because of insulin resistance occurring during this period [6]. Insulin resistance is partly attributed to several hormonal changes which some of them contribute simultaneously to bone loss [7, 8].

However, beside physiological changes of bone metabolism during pregnancy, it may be affected by pregnancy complications. Gestational Diabetes Mellitus (GDM) is one of the most important conditions which complicates up to 14% of pregnancies worldwide [9]. GDM patients are more frequently affected by changes in bone turnover during pregnancy.

Compared with healthy pregnancies, women with GDM are at increased risk of later development of type 2 diabetes mellitus [9]. A recently performed meta-analysis strongly supported an association between diabetes type 2 and increased risk of fracture in both men and women, though contradictory reports about bone mineral density among patients with diabetes type 2 are presented [10]. It seems that there are other mechanisms that lead to decrease in bone competence beside low density. Increased or alterations in collage glycosylation as a consequent of hyperglycemia is proposed as an alternative mechanism [11]. These metabolic disturbances in bone leading to decrease bone strength in type 2 diabetics which may contribute to low bone quality in GDM.

Regarding relatively short duration of pregnancy, its effects on bone metabolism may not easily be detected via bone densitometry. Bone markers demonstrating bone turnover are used in investigations on bone metabolism during pregnancy. Bone resorption markers have been previously studied [12, 13] and upsurge during first trimester has been seen; however, bone formation markers except Osteocalcin increase only in third trimester [12-14].

He objective of the present study was to evaluate the biomarkers of bone formation and resorption during pregnancy, assess bone turnover in pregnant women suffering from GDM and compare it with normal pregnancies.

Methods

A cross-sectional study was conducted on 695 pregnant women referred to five academic hospital clinics of the Tehran University of Medical Sciences (TUMS).

The pregnant women without previous history of diabetes mellitus who referred to the clinics for prenatal care during the first half of the pregnancy were considered eligible. The study protocol was approved by the research ethics committee of Endocrinology and Metabolism Research Center (EMRC), and the ethics committee of the Ministry of Health and Medical Education.

After interview, a general physical examination was performed by a physician and informed consent was taken. Fasting blood samples were drawn and centrifuged for 30 minutes. Samples were frozen at -80°C in hormone laboratory of the Endocrinology and Metabolism Research Center (EMRC).

Measurements

The patients were assessed via universal screening for GDM and 50-g oral glucose challenge test (OGCT) and 100-g oral glucose tolerance test (OGTT) were performed between the 24th and 28th weeks of pregnancy as previously described [6]. The previous medical history, obstetrics history, and family history of diabetes mellitus were taken.

Both serum calcium and phosphorus were measured by colorimetric using Kavoshyar enzyme kit (Kavoshyar, Iran) and Sheem enzyme kit (Sheem enzyme, Iran), respectively.

One of the markers of bone formation, Osteocalcin, was measured by immunoassay (ELISA) using a Bioscience kit (Nortic

Bioscience Diagnostic A/S, Denmark). The intra- and inter-assay CV were 2.6% and 4.7%, respectively. Another marker of bone resorption was the serum C-terminal telopeptides of type I collagen: serum Crosslaps. Crosslaps were measured by immunoassay (ELISA) using a Bioscience kit (Nortic Bioscience Diagnostic A/S, Denmark), with intra- and inter-assay CV of 5.1% and 6.6%, respectively.

Statistical analysis

Data were analyzed using SPSS software, version 11.5. The student's t-test was used to compare the differences between the means of variables. The Chi-square test was used to compare the frequency of variables. Pearson correlation was used to investigate correlation between two variables. In all the tests, level of significance was set at 0.05.

Results

Six hundred ninety five pregnant women were recruited. GDM was diagnosed in 7% ($n = 51$) of all participants. Mean values of age, pre-pregnancy BMI and number of parities were higher among GDM group in comparison with healthy pregnant women. Characteristics of the participants are summarized in Table 1. There was no significant difference between GDM patients and healthy pregnant women regarding gestational age.

Among GDM patients, fasting blood sugar (FBS), insulin and C-peptide levels were higher (Table 2). Assessing insulin resistance, the HOMA index also was significantly higher in GDM patients (Table 2). There was significant higher level of Osteocalcin and Crosslaps in GDM compared with normal group (for Osteocalcin 10.03 ± 5.94 *vs.* 5.29 ± 2.95, P= 0.001 and for Crosslaps 0.44 ± 0.21 *vs.* 0.21 ± 0.21, P= 0.001). No significant correlation was found between Osteocalcin and Crosslaps either in GDM or normal group (P= 0.7).

Univariate analysis after adjusting for body mass index (BMI) and age revealed that there was no independent relation between serum levels of Osteocalcin and GDM (P= 0.3); while, there was independent significant relation between serum levels of Crosslaps and GDM (P= 0.001).

The serum levels of Osteocalcin had no significant correlation with age, parity, pre-pregnancy BMI, gestational age and FBS, calcium (Ca), and HOMA index. Of note, the serum levels of Crosslaps had significant correlation with age (P= 0.04, r= 0.11), pre-pregnancy BMI (P= 0.02, r= 0.13), FBS (P= 0.001, r= 0.2), HOMA index (P= 0.01, r= 0.16) but not correlated with gestational age, and Ca.

Concerning bone markers and insulin resistance, after adjusting BMI and age, in regression model, the HOMA index had significant relations with serum levels of Crosslaps (P= 0.03, β= 0.16). In this case, there was no significant relation with Osteocalcin (P= 0.21, β= −0.29).

Table 1. Baselines characteristics of pregnant women

Maternal characteristics	GDM (n=51)	Non-GDM (n=644)
Age (years) [*][§]	30±5	25±5
Parity [**][§]	2(9)	2(6)
Gestational age (weeks) [*]	25±4	26±3
BMI (kg/m^2) [*][§]	28.2±4.9	24.9±5.4

[*] mean±SD
[**] Median (interquartile range)
[§] P-values were significant (P<0.05)

Table 2. Levels of maternal biochemical markers

Characteristics	GDM (n=51)	Non-GDM (n=644)
Fasting glucose(mmol/l) [*]	5.5±1.4	4.2±0.6
Fasting insulin (μIU/ml) [*]	21.6±18.1	13±9
Fasting c-peptide (ng/ml) [*]	3.7±4.8	1.6±2.3
HOMA index [*]	5.1± 4.5	2±1.6
Crosslaps (ng/ml) [*]	0.4±0.2	0.2±0.2
Osteocalcin (ng/ml) [*]	10±5.9	5.2±2.9

Data present as mean±SD; [*]P-values were significant (P<0.05).

Discussion

Bone formation and bone resorption are components of bone remodeling and an imbalance between the two is thought to cause most of the metabolic bone diseases [15]. Bone remodeling is consistent with corresponding blood biochemistry changes [5] and these biochemical markers are reliable indicators for bone turnover. Studies on bone metabolism during pregnancy suggest substantial bone turnover alteration [1, 16-18]. Rise in bone resorption markers including pyridinoline, deoxypyridinoline and N-telopeptide has been reported during first trimester by Black *et al.* [12, 13]. Among various bone resorption markers, Crosslaps are formed directly at the onset of type I collagen degradation. They are more sensitive markers of bone turnover than pyridinium crosslinks which are also released during type I collagen degradation [19]. Hellmeyer *et al.* detected in a longitudinal study [20] that serum level of Crosslaps also increased in second and third trimester. As well, Kaur *et al.* observed during a cohort study a significant increase in Crosslaps from the baseline by week 36th of pregnancy [21].

However, bone formation markers like bone-specific alkaline phosphatase (BALP) and PICP (carboxyterminal propeptide of type I collagen) remain unchanged until week 22-28th and then increase exponentially until term [12-14]. Exceptionally, Osteocalcin reported in one study decreased in pregnant women compared with non-pregnant controls [14]. In the present study, there was a significant higher level of Osteocalcin and Crosslaps in GDM compared with normal group. To our knowledge, only few data are available in GDM patients on maternal serum markers of bone formation and resorption. Ogueh *et al.* [22] reported a higher levels of ICTP (cross-linked carboxyterminal telopeptide of type I collagen) as a resorption marker and PICP at the time of delivery in GDM patients though it was not significant. Low sample size of their study (19 cases *vs.* 19 controls) may explain the insignificancy. Consistent with our findings, they showed a significant correlation between 1 hour postprandial blood glucose at week 26th of gestation and the maternal levels of PICP and ICPT.

We found a significant relation between GDM and serum levels of Crosslaps independent of BMI and age. Our finding is consistent with results reported by Suzuki *et al.* [23] clarifying the pathogenesis of altered bone metabolism in patients with type 2 diabetes mellitus. They showed that urinary excretion of Crosslaps significantly increased in the diabetics as compared with the controls. Also, another study comparing Crosslaps between type 2 diabetics and controls demonstrated its lower level among diabetic patient, but after adjusting the participants by sex, no difference was found between two groups among women [24].

Although, we found a higher level of Osteocalcin in GDM, it was correlated with GDM dependently of age and BMI. Oz *et al.* showed lower level of Osteocalcin in patients with type 2 diabetes of two sexes compared with healthy people [24]. Bouillon and colleagues suggested that hyperglycemia may

suppress osteoblast maturation [25]. On the other hand, since Osteocalcin is known to be glycosylated [26], this may affect the measurement of serum Osteocalcin level. So, measuring formation markers other than Osteocalcin such as PICP or BALP may produce more reliable results for bone formation assessment in hyperglycemic status like GDM.

We found significant relations between serum level of Crosslaps and HOMA as an indicator of insulin resistance independent of BMI and age. As mentioned above, in patients with type 2 diabetes, both increase and decrease of Crosslaps has been reported [23, 24]. Overall bone metabolism changes in hyperglycemic and hyperinsulinemic status is controversial [10, 11]. It has been hypothesized that developing insulin resistance resulted from changes in the metabolic pathways does not generalize to bone metabolism [27]; accordingly, and regarding that both osteoblast and osteoclast express insulin receptors [28]; increase in cumulative insulin exposure due to postprandial hyperinsulinemia may cause osteoblast and osteoclast overactivity, leading to exaggerated bone turnover in GDM. Given higher bone turnover, its trend to either formation or resorption expedites bone gain or loss. More surveys are needed to confirm it in vitro and in vivo.

Besides, we found no relation between Osteocalcin and GDM independent of BMI. The bone remodeling cycle begins by mechanism which mediated by cells of the osteoblast lineage. The interaction between the receptor activator of NF-kappa B ligand (RANKL) and a receptor on osteoclast precursors called RANK, results in activation, differentiation, and fusion of hematopoietic cells of the osteoclast lineage; so that, they begin the process of resorption [28]. Regarding independent relation between Crosslaps and GDM, we suggest that osteoclast hyperactivity is not related just to osteoblast stimulation and their overstimulation result from factors which my play a role in pathogenesis of GDM. It is in harmony with recent proposed common

mechanisms in osteoporosis and metabolic syndrome [29] which is known as a risk factor for type2 diabetes [30]. Recently, it has been reported that both diabetes and metabolic syndrome, independently of age and BMI, are associated with higher frequency of osteoporosis and lower frequency of BMD of hip and are risk factors for increased incidence of hip fractures in men [31].

Reviewing other results of our study about Crosslaps, we found weak correlation between Crosslaps and components of GDM like HOMA. It is in accordance with our explaining that GDM and osteoclast hyperactivity share some causative factors and increased bone resorption is not consequence of GDM or insulin resistance. Further studies are needed to clarify this hypothesis.

Namgung and Tsang [32] concluded in their review of several studies on bone in the pregnant women that elevating formation markers beside increase in resorption markers observe in third trimester. They stated that uncoupling of bone resorption from formation could contribute the fetal requirement for calcium while maintaining maternal calcium homeostasis and lead to a net loss of maternal bone. Regarding our findings, hyperactivity of osteoclast leading to bone resorption in GDM may suggest bone remodeling in favor of bone loss in mothers with gestational diabetes. Further longitudinal studies comparing bone marker and bone density in women with and without GDM are warranted during preconception and postpartum phases for precise clarifying.

In conclusion, our study showed a higher incidence of bone turnover in GDM patients during pregnancy; specially, increased bone resorption which was independently correlated with GDM proposed common pathologic pathways leading to GDM and osteoporosis.

Acknowledgement

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