Original Article

A study of bone turnover markers in gestational diabetes mellitus

Sheelu Shafiq Siddiqi, Abhijit Girish Borse[1], Anjum Pervez[1], Shaheen Anjum[2]

Rajiv Gandhi Centre for Diabetes and Endocrinology, J. N. Medical College and Hospital, Departments of [1]Medicine and [2]Gynaecology, J. N. Medical College and Hospital, Aligarh, Uttar Pradesh, India

ABSTRACT

Introduction: Gestational diabetes is defined as carbohydrate intolerance resulting in hyperglycemia of variable severity with the first recognition during pregnancy. Established risk factors for gestational diabetes mellitus (GDM) are maternal age, obesity, family history of diabetes, etc. Vitamin D, parathyroid hormone (PTH), and various other hormones are known for their function in maintaining calcium and phosphorous homeostatic. Furthermore, Vitamin D, PTH serum ionized calcium, and alkaline phosphatase (ALP) have been reported to be altered with glucose homeostasis. The present study compares the bone markers in pregnant women with and without gestational diabetes. **Materials and Methods:** This cross-sectional study was conducted at outpatient antenatal check-up clinic and outpatient diabetic clinics at J. N. Medical College and Hospital, Aligarh. One hundred pregnant females, of which fifty with GDM and fifty without GDM, were included in the study from January 2014 to November 2015. Detailed history, physical examination, and anthropometric measurement were done. Bone turnover markers in the form of Vitamin D, parathyroid hormone, serum ionized calcium, and serum ALP were measured in pregnant women who had gestational diabetes which was compared with normal pregnant women. **Results:** In our study, the mean age of participate of GDM group was 28.2 ± 3 years, while the mean age group in non-GDM group was 25.44 ± 2.78 years. Ionized calcium in GDM was found to be 4.606 ± 0.354 mEq/L, while in non-GDM, it was 4.548 ± 0.384 mEq/L, P = 0.430. Vitamin D came out to be 21.80 ± 9.48 ng/ml, while it was 32.346 ± 8.37 ng/ml in non-GDM group. Serum PTH in GDM group was 71.436 ± 36.189 pg/ml and 37.168 ± 8.128 pg/ml in nondiabetic gestational group. Serum ALP in GDM group was 9.1 ± 4.56 KA U/dl and 6.98 ± 2.2 KA U/dl in nondiabetic gestational group, P - 0.0038. In GDM group, there was a significant negative linear correlation between PTH and 25-hydroxyvitamin D with research correlation coefficient r = –0.9073, P = 0; there was a significant positive linear correlation coefficient between PTH and ALP with Persian correlation coefficient r = 0.6597, P = 0; there was no statistically significant correlation between PTH and ionized calcium r = 0.1416, P = 0.3267. **Conclusion:** All GDM subjects should ideally be screened for serum calcium, vitamin D, PTH, ALP. If found impaired should immediately be corrected in order to prevent its adverse effects on maternal and fetal outcome. Vitamin D supplementation should ideally be initiated in all GDM females even if the above parameters are not investigated in Indian setup.

Key words: Alkaline phosphatase, gestational diabetes mellitus, king armstrongs unit

Introduction

Gestational diabetes is defined as carbohydrate intolerance resulting in hyperglycemia of variable severity with the first recognition during pregnancy.[1] The frequency, if gestational diabetes mellitus (GDM), usually reflects the frequency of Type 2 diabetes mellitus in the underlying population.[2,3] Established risk factors of GDM are maternal age, family history of diabetes.[4] Decreased physical activity and adoption of a modern lifestyle in developing countries may

Corresponding Author: Dr. Sheelu Shafiq Siddiqi,
Rajiv Gandhi Centre for Diabetes and Endocrinology,
J. N. Medical College and Hospital, Aligarh, Uttar Pradesh, India.
E-mail: shafiqsheeluims@gmail.com

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all contribute to increase in the prevalence of GDM.[5] Infant of women with GDM or diabetes are at increased risk of developing obesity, impaired glucose tolerance and diabetes as children or young adult.[6,7]

The effect of pregnancy on the maternal skeleton has preoccupied the scientific medical community for decades. Pregnancy and lactation increase calcium demand for fetal skeleton development and mild production, respectively, and bone serves to supply calcium during these reproductive periods.[8] It is considered that pregnancy could affect peak bone mass and accelerate the risk of developing osteoporosis in later stages of life. Biochemical markers of bone turnover increase slowly during pregnancy, with the highest increase measured in the third trimester.[9,10] A significant increase is also observed in bone formation and restoration leading to bone turnover and fetal development.

Two resorption markers, carboxy-terminal collagen cross-links and N-telopeptide cross-link increase steadily throughout pregnancy, with the largest occurring between the second and third trimesters.[11] Markers of bone formation also increase during pregnancy but follow a different pattern of change; for example, procollagen Type 1 carboxy-terminal propeptide and bone-specific alkaline phosphatase (ALP) vary little during the first trimester but increase significantly (44%) between the second and third trimesters.[12] Hormonal factors produced during pregnancy could cause changes in bone turnover because of their growth hormone like effects.

Bone loss during pregnancy and lactation may result in pregnancy/lactation-associated osteoporosis which manifests as back pain, height loss, or vertebral fractures.[10,13] A recent densitometry study on GDM women revealed a reduction in vertebral bone mineral density when compared with nondiabetic pregnant women;[14] it has been reported that greater than normal bone loss is present in 40% of GDM women within 3 months of postpartum.[15]

The production of Vitamin D is regulated by parathyroid hormone (PTH). The active form, 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), is also produced by placenta during pregnancy with possible autocrine or paracrine function. Maternal status of Vitamin D has a direct effect on fetal growth through insulin.[16] There is uncoupling of Vitamin D metabolism from calcium during pregnancy such that serum $1\text{-}25(OH)_2D$ level become more than double at the end of the first trimester as compared during nonpregnant state without a concurrent change in serum calcium concentration.[17] Studies have also evaluated the role of Vitamin D as an immunomodulation, action of insulin, inhibiting angiogenesis or inflammatory cells.

Normalization of serum PTH and calcium is a useful end-point in determining successful Vitamin D supplementation in normal pregnancy. According to some studies, total $1,25(OH)_2D$ alone is a better measure of maternal Vitamin D status because PTH threshold estimates cannot be defined precisely.[18-20] PTH levels have been found to be low-normal in the serum of pregnant women in all three trimesters.[21-25] Five prospective, longitudinal studies found that the mean PTH level was in the low-normal range (i.e., 50% of the mean nonpregnant value) during the first trimester but increased steadily to the mid-normal range by the end of pregnancy.[26-29]

Fetus receives calcium from maternal blood though placenta to maintain fetal bone growth. During pregnancy changes occur in maternal compartment that permits calcium accumulation by doubling intestinal absorption by 12th week of gestation[30] and most likely consequences of increased 1-25(OH)2D concentration.[31] Approximately 200 mg/day of calcium is deposited in the fetal skeleton during the third trimester[32] with a net accumulation of 25–30 g.[33] The hypercalciuria of pregnancy is of mostly due to a combination of increased gastrointestinal absorption of calcium and an increased glomerular filtration rate.[34] Despite the increased transfer of calcium to the fetal skeleton, and increased urinary calcium excretion, there is surprisingly litter evidence that pregnant women suffer from skeleton demineralization. Throughout gestation, calcium and phosphate levels in fetus increase steadily, further at term, it increases the maternal level although the level of ionized calcium in maternal serum remains unchanged, however, total calcium and phosphorous declines.[35,36]

Maternal adjustments in calcium metabolism occur during pregnancy compensate, at least in part, for fetal calcium demands and urinary losses. In particular, levels of 1,25-dihyfroxyvitamin D are 2–3 times the levels in nonpregnant women, with an increase in intestinal calcium absorption from 0.8 to 1.5 g/day reported.

Some observational studies have shown an inverse association between Vitamin D and calcium status and insulin resistance.[37,38]

Results from randomized trials on the effect of Vitamin D and/or calcium supplementation on insulin resistance show neither effect[39,40] nor improvement of insulin action with supplementation.[41,42] Women supplemented with calcium and Vitamin D experienced a significant decrease in fasting glucose and insulin levels.[43]

The study has reported that there is an increase in activity of bone isoenzyme of serum ALP of 52%, 82%, and 72%

of diabetic patients on dietary, oral agents, and insulin agents, respectively, and a significant positive correlation between the activity of bone isoenzyme and urinary hydroxyproline excretion in diabetes to be similar to osteoporosis.[44] The influence of blood glucose levels in the expression of the biochemical parameters of bone metabolism was most pronounced in patients or oral agents. The result explained moderate hyperphosphatasia, known in diabetes, and supported a metabolic etiology for the bone disease in diabetes mellitus.[44]

Materials and Methods

This cross-sectional study was conducted at outpatient antenatal check-up clinic and outpatient diabetic clinics at J. N. Medical College and Hospital, Aligarh. One hundred pregnant female between 20 and 40 years of age with 24 weeks of gestation until full-term with no history diabetes mellitus were enrolled during January 2014 to November 2015. Detailed history, physical examination, and anthropometric measurement were done. Bone turnover markers in the form of Vitamin D, parathyroid hormone, serum ionized calcium, and serum ALP were measured in pregnant women who had gestational diabetes which was compared with normal pregnant women. The screening glucose challenge test was performed between 24 and 28 weeks. No previous fasting was required for this screening test, in contrast to oral glucose tolerance test (OGTT). Women were given a solution containing 50 g of glucose and measured blood level 1 h later. The cutoff point was set at 140 mg/dl.

- Those pregnant women showed negative result to screening test were considered as control group (Group A)
- Those pregnant women turn positive to screening test were subjected to OGTT.

Oral glucose tolerance test

The OGTT performed in the morning after overnight fast of between 8 and 14 h. During the 3 previous days, the subject was instructed to have an unrestricted diet (containing at least 150 g carbohydrate/day) and unlimited physical activity. The subject remained at rest during the test.

The test involved drinking a solution containing a 100 g of glucose, and samples were drawn at the start, 1, 2, and 3 h after oral glucose load.

Around 1.5–2 ml blood sample was drawn from antecubital vein fluoride bulb.

Those women who satisfy the criteria of GDM on the basis of OGTT were included in the study (Group B).

Estimation of ionized calcium, Vitamin D, 25(OH)D, intact PTH (iPTH) and Alkaline phosphatase (ALP).

Calcium estimation

The sample was collected in 20 cc glass syringe, centrifuged at 4°C at 3000 rpm, for 5 min, and kept at 20°C until processed. Ionized calcium was estimated serum using ion selective electrode analyzer, on Cobas 121 analyzer (Roche Diagnostics, Switzerland), with an intra-assay coefficient of variation 2% and inter-assay coefficient of variation 3%.

Expected normal range was 4.1–5.6 mEq/L.

Estimation of serum parathyroid hormone

The PTH was estimated by radioimmunoassay; the determination of PTH is a "sandwich-" type assay. Two antibodies, directed against different epitopes of the PTH molecule and not competing with each other, were used in the kit. The samples and calibrators were incubated in tubes coated with a polyclonal antibody. Following the first incubation, the content of the tubes was aspirated carefully. A solution containing the second monoclonal antibody, ^{125}I-labeled, was taken and added to each tube. Following a second incubation, the content of tubes was aspirated, and after washing twice, bound radioactivity was measured. Unknown values were determined as PTH in the sample was directly proportional to radioactivity.

- Precision: Intra-assay coefficient of variation was found to be ≤12.2%. Inter-assay coefficients of variation were found to be ≤9.7%
- Expected value: As an indication, values between 17.3 and 72.9 pg/ml were considered as the normal range.

Estimation of serum 25-hydroxyvitamin D

The 25(OH)D was estimated using chemiluminescence-immunoassay from maternal serum using DiaSorin Liaison automated analyzer. The liaison 25(OH)D total assay is a direct competitive chemiluminescence immunoassay for human serum or plasma. The assay uses magnetic particles (solid phase) coated with antibody against 25(OH)D and 25(OH)D conjugated to an isoluminol derivative (tracer). During the first incubation phase (10 min), 25(OH)D was dissociated from binding protein by buffer containing 10% ethanol and then bounds to the anti-25(OH)D antibody on the solid phase. After a second 10 min incubation with the tracer, the unbound material was washed off, and starter reagents were added to generate a flash chemiluminescent signal related to 29(OH)D concentration.

- Precision: Intra-assay coefficient of variation was found between 2.9% and 5.5% and inter-assay coefficient of variation was found between 6.3% and 12.5%
- The participants from both groups were classified into the following groups based on values of Vitamin D based on recommendations of endocrine society guidelines 2011, (Holick: 2007, Heaney: 2004, Malabanan *et al*.: 1998)
- 25(OH)D excess: >150 ng/dl
- Normal: 30–100 ng/dl
- Insufficiency: 21–29 ng/dl
- Deficiency: <20 ng/dl.

Estimation of serum alkaline phosphatse

ALP at an alkaline pH hydrolyses di sodium Phenylphosphate to form phenol. The Phenol formed reacts with 4- Aminoantipyrine in the presence of Potassium Ferricyanide, as an oxidising agent, to form a red coloured complex. The intensity of the colour formed is directly proportional to the activity of ALP present in the sample .This was determined by digital photo colorimeter METZ-401M.

Expected normal range was 4–13 KA U/dl.

Statistical analysis

Data were expressed as mean ± standard deviation and $P < 0.05$ was considered statistically significant. Baseline characteristic between the groups without gestational diabetes (Group A) and with gestational diabetes (Group B) was analyzed by paid *t*-test, and the correlation of PTH with body mass index (BMI), 25(OH)D, ALP, serum ionized calcium was assessed by Pearson's test using SPSS version 19 (IBM, New York, United States).

Results

In our study, the mean age of participate of GDM group was 28.2 ± 3 years, while the mean age group in non-GDM group was 25.44 ± 2.78 years. BMI measured in GDM group was 23.8976 ± 2.55 kg/m^2 and 26.21 ± 3.47 kg/m^2 in non-GDM pregnant females. Ionized calcium in GDM was found to be 4.606 ± 0.354 mEq/L while in non-GDM was 4.548 ± 0.384 mEq/L, $P = 0.430$. Vitamin D came out to be 21.80 ± 9.48 ng/ml, while it was 32.346 ± 8.37 ng/ml in non-GDM group. Serum PTH in GDM group was 71.436 ± 36.189 pg/ml which was 37.168 ± 8.128 pg/ml in nondiabetic gestational group. Serum ALP in GDM group was 9.1 ± 4.56 KA U/dl and 6.98 ± 2.2 KA U/dl in nondiabetic gestational group, P - 0.0038 [Table 1]. In GDM group, there was a significant negative linear correlation between PTH and 25(OH)D with research correlation coefficient $r = -0.9073$, $P = 0$; there was a significant positive linear correlation coefficient between PTH and ALP with Persian correlation coefficient $r = 0.6597$, $P = 0$; there was no statistically significant correlation between PTH and ionized calcium $r = 0.1416$, $P = 0.3267$ [Table 2].

Discussion

In our study, in Group B (females with GDM), 27 participants out of 50 (54%) were obese, whereas in Group A, 13 participants (26%) were obese. The participants in Group B had significantly higher BMI as compared with participants in Group A, and among the participants in Group B (females with GDM), there was statistically significant negative linear correlation between 25(OH)D and BMI. This suggests an association of obesity with hypovitaminosis D as well as GDM.

Many of the previous studies had reported obesity as a well-known risk factor for 25(OH)D deficiency.[45,46] An analysis of the National Health and Nutrition Examination Survey (NHANES) 2003–2004 data also demonstrated that 25(OH)D deficiency was highly prevalent in overweight and obese American subjects (The NHANES 2003/2004).

Vitamin D plays a great role in bone metabolism and mineral homeostasis. Vitamin D insufficiency has a known effect on bone density, neonatal Vitamin D and calcium status, and childhood rickets.[47]

In our study, in Group B (females with GDM), 24 out of 50 participants (48%) had deficient Vitamin D, whereas in Group A, 5 out of 50 participants (10%) had deficient Vitamin D, indicating increased frequency of hypovitaminosis D among Group B participants [Table 3]. This suggests that there is a significant difference in 25(OH)D level between diabetes and nondiabetic pregnant females.

Maghbooli *et al*. demonstrated that maternal serum levels of 25(OH)D during 24–28 weeks of pregnancy were significantly lower in women with GDM compared with pregnant females without GDM.[48]

In our study, in Group B (females with GDM), 23 participants out of 50 (46%) had raised PTH, whereas the level of PTH is normal in Group A (females without GDM) [Table 4].

A previous study suggested that iPTH levels to be significantly higher in pregnant women with severity deficient 25(OH)D levels compared to those with

Table 1: Baseline participant characteristic and biochemical parameters

Variables	Pregnant females without gestational diabetes (Group A)	Pregnant female with gestational diabetes (Group B)	*P*
Age (years)	25.44±2.78	28.2±3	<0.001
BMI (kg/m^2)	23.8976±2.5509	26.2108±3.4766	0.0003
HGB (g/dl)	11.3±0.72	11.0±0.87	0.619
TLC/mm^3	6098±1091.8	6220±1525.97	0.64
PLT/mm^3	241,140±51,086.6	223,640±64,417.9	0.1355
Blood urea (mg/dl)	27.28±3.844	25.916±5.911	0.1745
Serum creatinine (mg/dl)	0.837±0.1532	0.8024±0.1349	0.2336
ALP (KA U/dl)	6.98±2.2	9.1±4.56	0.0038
Ionized Ca$^+$ (mEq/L)	4.548±0.384	4.606±0.354	0.430
Vitamin D (ng/ml)	32.346±8.378	21.808±9.486	0<0.0001
PTH (pg/ml)	37.168±8.128	71.436±36.189	<0.0001

BMI: Body mass index, ALP: Alkaline phosphatase, TLC: Total leukocyte count, PLT: Platelet, PTH: Parathyroid hormone, HGB: Hemoglobin

Table 2: The correlation analysis of parathyroid hormone with 25(OH)D , ionized calcium, alkaline phosphatase, body mass index in Group B (gestational diabetes mellitus females)

Variables	PTH		
	r	*P*	Significance
25(OH) D	−0.9073	0	S
ALP	0.6597	0	S
Serum ionized calcium	0.1416	0.3267	NS
BMI	0.8037	0	S

BMI: Body mass index, ALP: Alkaline phosphatase, PTH: Parathyroid hormone, 25(OH) D: 25-hydroxyvitamin D, S: Significant, NS: Not significant

Table 3: Classification of Participants on basis of Vitamin D Levels

	Group A (%)	Group B (%)
Sufficient	32 (64)	12 (24)
Insufficiency	13 (26)	14 (28)
Deficiency	5 (10)	24 (48)
Total	50 (100)	50 (100)

Table 4: Frequency of Hyperparathyroidism in both groups

	Group A (%)	Group B (%)
Increased PTH level	0	23 (52)
Normal PTH level	50 (100)	24 (48)
Total	50 (100)	50 (100)

PTH: Parathyroid hormone

insufficient and sufficient 25(OH)D levels and iPTH concentrations were significantly higher in women with GDM as compared to controls.[49] Some studies had reported that increased PTH levels, either primary or secondary to other disorders, were associated with impaired glucose tolerance.[50-52]

In our study, ALP in participants of Group B (females with GDM) was raised as compared with Group A (females without GDM).

Few studies in past had reported raised alkaline phosphatase in diabetes, attributed to bone disease. Heazell *et al* 2006 reported a case of pregnancy complicated by gestational diabetes and hypertension in which extremely high elevation of bone specific isoenzyme of alkaline phosphatase was noted during labour this lead to elevation in total serum alkaline phosphatase which couldn't be assume to arise from placenta.[53,54]

In our study, serum ionized calcium was in normal range in both groups with no statistically significant difference between both groups.

In our study, participants of Group B (females with GDM) had increased PTH levels, reduced 25(OH)D levels, increased ALP, and more obesity in comparison to participants in Group A. While serum ionization calcium was comparable in both groups as well as in Group B, PTH showed statistically significant negative linear correlation with 25(OH)D and statistically significant positive linear correlation with ALP and BMI.

In this study, there were significant increased bone turn markers in participants of Group B than those of Group A. Significant number of females with GDM had secondary hyperthyroidism; more survey is needed to confirm it *in vitro* and *in vivo*.

There are limited studies on the status of bone turnover markers in GDM; more studies are required from India, in view of rising trend of diabetes. Our study has demonstrated strong correction between bone turnover marker and GDM. Studies with large sample size are needed to further demonstrate the status of bone turnover markers in GDM.

Conclusion

The study shows a high prevalence of obesity, hypovitaminosis D, and hyperparathyroidism, in female with GDM, in comparison to females without GDM. This justifies that all pregnant women should be investigated for bone turnover markers and corrected well in time to bring about good maternal and fetal health outcome. Other newer bone markers which do not interfere with glycosylation may be considered to attain bone health estimation.

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Conflicts of interest

There are no conflicts of interest.

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