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Original Article

Association Between Gestational Diabetes Mellitus and Maternal Bone Metabolism: A Cross-Sectional Study

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ABSTRACT

Gestational diabetes mellitus (GDM) is a prevalent metabolic condition complicated by pregnancy that relates to poor maternal and infant outcomes. The connection between glucose intolerance and a shift in the bone metabolism in pregnant women is a developing field whose applicability of bone turnover measurements in GDM is yet to be determined. **Objectives:** To determine the relationship between GDM and maternal bone turnover indices and GDM predictors. **Methods:** The groups of pregnant women with GDM and those without diabetes were 120 and 60, respectively, in the second trimester of this cross-sectional study. Serum osteocalcin, a cross-linked C-telopeptide of type I collagen (CTX), and bone-specific alkaline phosphatase (B-ALP) were measured. It comprised a series of clinical, biochemical, and obstetric data, such as the body mass index (BMI) and insulin resistance, which was determined using the homeostasis model assessment (HOMA-IR). ROC curve analysis and logistic regression analysis were carried out. **Results:** GDM women were significantly older women with much higher BMI and HOMA-IR compared to controls ($p<0.001$). B-ALP level of the GDM group was substantially low ($p<0.05$), and CTX did not change. The results of the logistic regression analysis identified the independent predictors of GDM as osteocalcin, BMI, and HOMA-IR (OR: 0.565; 0.442-0.722; OR: 1.309; 1.062-1.614; OR: 2.289; 1.090-4.805). The discriminative power (AUC = 0.905; $p<0.001$) was found to be powerful. **Conclusions:** GDM is also related to bone metabolism, osteocalcin, BMI, and HOMA-IR are independent predictors.

INTRODUCTION

Approximately 7–14% of pregnant women worldwide develop GDM, which is characterized by glucose intolerance during pregnancy [1]. The prevalence of GDM in China and Asia is reported to be 14.8% and 14%, respectively [2, 3]. GDM upsurges the risk of obstetric complications such as preterm delivery, macrosomia, neonatal metabolic disturbances, and cesarean birth [4]. Insulin resistance and dysfunction of pancreatic β-cells are central to the pathogenesis of GDM, though recent work has increasingly implicated osteocalcin and other bone-derived hormones in regulating energy metabolism and glucose homeostasis [5]. Although once regarded

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primarily for its structural role, recent studies have revealed that the skeleton also exerts essential regulatory effects on metabolic functions [6]. Osteocalcin, a non-collagenous protein secreted predominantly by osteoblasts in bone, has been increasingly recognized not just for its structural role in mineralization, but also for important endocrine functions in energy metabolism. In its fully γ-carboxylated form, osteocalcin binds to hydroxyapatite in bone; however, an undercarboxylated fraction (ucOC) released during bone resorption appears to be the hormonally active form that impacts glucose and lipid homeostasis [7]. The ucOC enhances beta cell proliferation, enhances insulin secretion, and develops insulin sensitivity in adipose tissue and muscles, and thus lessens fat mass as proved in animal studies. Also, insulin resistance develops in osteocalcin-deficient mice; osteocalcin administration reverses these metabolic derangements [8]. Despite strong preclinical evidence, several gaps remain unexplored. For example, the human studies do not differentiate between ucOC vs carboxylated fraction of osteocalcin. Furthermore, the studies omit concurrent measurement of other turnover makers like B-ALP, CTX, and HOMA-IR [9, 10]. Notwithstanding, these markers are often used to measure changing processes in bone growth and bone resorption.

Limited human evidence exists regarding the relationship between gestational diabetes mellitus (GDM) and maternal bone metabolism, particularly the role of bone turnover markers such as osteocalcin, CTX, and B-ALP in predicting metabolic dysfunction during pregnancy. Most previous studies have either focused on general glucose regulation or failed to assess multiple bone biomarkers alongside insulin resistance parameters, leaving uncertainty about their combined clinical relevance. This gap creates a challenge for early identification of metabolic alterations that may influence maternal and fetal outcomes. Therefore, this study aimed to examine the association between GDM and selected bone turnover markers in second-trimester pregnant women to better understand their potential as indicators of metabolic risk.

METHODS

The study was a cross-sectional one conducted in the University of Lahore Teaching Hospital (ULTH) in the period between January and December 2023. Ethical approval was received by the Institutional Research Ethics Committee (IREC) of the Department of Pharmacy, University of Lahore (No. IREC-2023-30H), and informed written consent was taken from all the subjects as per the institutional ethics. The research was done in accordance with the Declaration of Helsinki. The sample size was calculated by Open Epi with a 95 percent level of confidence, the power was 80 percent, and the anticipated differences in osteocalcin levels as reported in the literature were 108; 120 women were recruited to cover dropouts. The study sample was recruited sequentially in the normal antenatal care, with 60 having GDM and 60 having normal glucose tolerance (frequency-matched on ages and gestational age). The inclusion criteria were uncomplicated singleton pregnancies of 2428 weeks of gestation with ultrasound (Aplio 300 system, Canon Medical, Japan) transvaginal (PVT-375BT) and abdominal (PVT-475BT) transducer (16 MHz). A 75g OGTT: fasting glucose >95mg/dL, 1h>180mg/dl, or 2h 153mg/dl was used to diagnose GDM based on IADPSG criteria. Women who had pre-gestational diabetes, thyroid conditions, chronic kidney or liver disease, or women taking drugs that influence the bone or glucose metabolism were excluded. Maternal age, BMI, blood pressure, and obstetric history were taken. Plasma glucose, insulin, lipid profile, and bone turnover markers. Venous blood plasma samples were collected by means of fasting. Enzymatic measurements were performed to determine glucose and lipids (Roche Diagnostics, Germany), insulin through chemiluminescent immunoassay (Abbott Laboratories, IL, USA), and HOMA-IR as [fasting insulin (μIU/mL) × fasting glucose (mg/dL)]/ 405. Measurement of bone markers was done via ELISA: osteocalcin (Immunodiagnostic Systems, UK), B-ALP (MicroVue BAP EIA, Quidel, USA), and CTX (Serum CrossLaps ELISA, IDS, UK). IBM SPSS version 26.0 was used to conduct statistical analysis. Continuous data are mean and SD, and categorical data are in the form of frequencies and percentages. The difference between groups was measured using the 2x2 test, and the Pearson correlation coefficient was employed to establish the independent predictors of GDM. Significance was set at $p<0.05$.

RESULTS

Continuous variables, including maternal age, BMI, fasting glucose, osteocalcin, B-ALP, CTX, and HOMA-IR, were summarized using descriptive statistics. The measured values were within the range of values anticipated in adult female and generally consistent across the subjects. These variables did not miss any data. Out of the 120 participants, there were 60 who were diagnosed with GDM, and 60 who were non-GDM controls, so it was possible to compare the two similar groups of 60 each. About 61.7 per cent of women gave birth via cesarean section. The proportion of neonatal hypoglycemia was found in 52/60 (87.5) infants born to mothers with GDM and 3/60 (5) non-GDM, which is associated with a corrected calculation (Table 1).

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Table 1: Descriptive Statistics of Maternal and Biochemical Parameters Among Study Participants(n=120)

Parameters	Values
Age	29.3 ± 3.7 Years
BMI	26.8 ± 2.9 kg/m^2
Fasting Glucose	91.7 ± 12.1mg/dL
Osteocalcin	11.9 ± 3.0 ng/mL
B-ALP	22.9 ± 4.6 U/L
HOMA-IR	2.3 ± 0.8
CTX	0.5 ± 0.1 pg/mL

An association was found between GDM and delivery mode. Women with GDM were more likely to deliver their babies by cesarean(61.7%%)than women without GDM(38.3%)(χ^2(1)= 5.08, p=0.024). Thus, there was a strong correlation between GDM and neonatal hypoglycemia. Among babies born to women with GDM, hypoglycemia was reported in 87.5%, whereas only 12.5% of babies in the non-GDM group were affected (χ^2 (1) = 9.22, p=0.002). Results suggest that maternal glycemic control may be associated with the baby's metabolic health(Table 2).

Table 2: Descriptive Statistics of Cesarean Delivery and Neonatal Hypoglycemia Among Women with Gestational Diabetes Mellitus

Variables	Category	Frequency (%)
GDM	Yes	60 (50 %)
	No	60 (50 %)
Cesarean delivery	Yes	74 (61.7 %)
	No	46 (38.3 %)
Neonatal Hypoglycemia	Yes	105 (87.5 %)
	No	15 (12.5 %)

The association between osteocalcin and key glycemic indicators was assessed using Pearson correlation coefficients. Lower osteocalcin levels were correlated with higher fasting blood glucose, and a significant negative correlation was observed between osteocalcin and insulin resistance (HOMA-IR). These findings suggest that reduced osteocalcin may be linked to adverse glycemic regulation in pregnant women(Table 3).

Table 3: Pearson Correlation of Osteocalcin with Fasting Glucose and HOMA-IR in Pregnant Women

Variables	Fasting Glucose Levels	HOMA-IR
Osteocalcin	r = 0.474**	r = 0.396**
	p<0.001	p<0.001

**Pearson correlation coefficient. p<0.001 (2-tailed) indicates statistical significance.

To compare biochemical markers and maternal characteristics between women with and without GDM, an independent sample t-test was used. Maternal age showed no significant difference between the two groups (p=0.544). However, women diagnosed with GDM demonstrated increased insulin resistance and poorer glucose control, reflected by higher BMI (p<0.001), fasting glucose (p<0.001), and HOMA-IR (p<0.001) relative to the control group. Differences were also observed in serum bone turnover markers. Osteocalcin levels were significantly lower in women with GDM compared with those without GDM (10.07 vs. 13.91 ng/mL, p<0.001). Bone-specific alkaline phosphatase (B-ALP) levels were modestly but significantly decreased in the GDM group (p=0.025), while CTX levels showed no significant difference between the groups(p=0.131)(Table 4).

Table 4: Descriptive Statistics of Maternal Age, BMI, Fasting Glucose, and Osteocalcin in Women with and without Gestational Diabetes Mellitus

GDM	Age	BMI	Fasting Glucose	Osteocalcin
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
No	29.15 ± 3.709	25.60 ± 2.292	83.04 ± 8.120	13.906 ± 1.2
Yes	29.57 ± 3.825	28.06 ± 3.055	100.40 ± 8.996	10.073 ± 0.9

*SD means Standard deviation

Binary logistic regression analysis was performed to evaluate the relationship between clinical and biochemical variables and GDM. BMI, osteocalcin, B-ALP, CTX, and HOMA-IR were the predictor variables in the model. The general model was statistically significant, 5 (5) = 77.79, p<0.001, which showed that the variables were useful in distinguishing between women with and without GDM. Cox and Snell R 2 and Nagelkerke R 2 indicated that the model explained 47.7% and 63.6% of the variance in GDM status, respectively. The Hosmer-Lemeshow goodness-of-fit test (2 = 14.70, p=0.065) showed that the model fitted the data adequately. The results indicated that a higher BMI(p=0.011, OR = 1.31), lower osteocalcin levels (p<0.001, OR = 0.57), and higher HOMA-IR (p=0.029, OR = 2.29) were independent predictors of GDM. On the other hand, there were no strong associations found between B-ALP and CTX in the final model. These results signify that insulin resistance, osteocalcin, and BMI are significant and independent variables that are connected with the threat of developing GDM. The logistic regression analysis produced a receiver operating characteristic (ROC) curve that illustrated excellent discrimination power in women with and without GDM. The curve was steeply rising towards the upper-left part, indicating a high sensitivity and specificity at different cutoff points. Generally, the model performed well with an AUC of 0.911. The Area Under the Curve Is 0.911, Indicating Excellent Predictive Performance(Figure 1).

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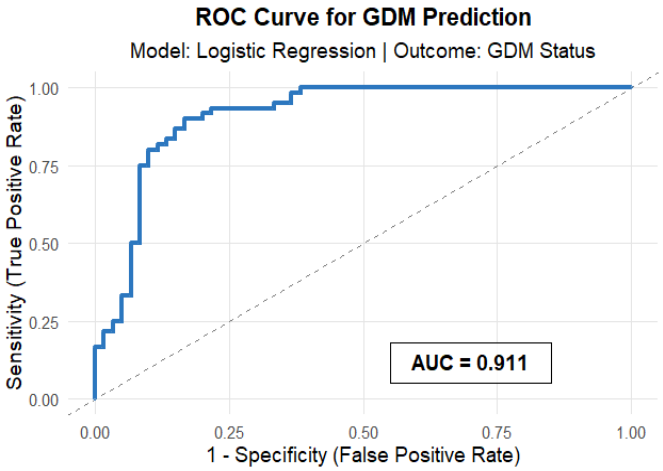

Figure 1: Receiver Operating Characteristic (ROC) Curve for the Logistic Regression Model Predicting Gestational Diabetes Mellitus Status

DISCUSSION

The effect of GDM on biomarkers of bone turnover, like osteocalcin, CTX, and B-ALP, was investigated in the current study. The findings revealed a significant alteration in these biomarkers compared to women without GDM. Thus, it suggests that there exists a potential association between impaired bone metabolism and glycemic control. During normal pregnancy, a steady decline in insulin activity is logical as it increases serum glucose level, thus ensuring a healthy supply of glucose to the fetus, especially during the second and third trimesters [11]. Thus, such a form of insulin resistance is considered natural and healthy for the fetus. During GDM, conversely, the pancreatic beta cells endue insufficient compensation towards serum glucose, which leads to maternal hyperglycemia [1, 12]. It is well known that bone-derived biomolecules are stimulated during the process of osteogenesis, and these molecules are involved in energy metabolism, consequently extending the role of bone beyond structural support. By the way, osteocalcin, a non-collagenous protein secreted by osteoblasts, has been reported to stimulate β-cell proliferation, improve insulin sensitivity, and increase insulin secretion in peripheral tissues [13]. Therefore, any physiological change in bone turnover, as seen during pregnancy, may affect glucose homeostasis and osteocalcin levels [14, 15]. The findings of the current study endorse the previous observations, as glucose tolerance and insulin resistance were obvious in the GDM patients [14]. Nevertheless, the physiological implications of this finding are complex enough, as osteocalcin exists in both uncarboxylated (ucOC) and carboxylated form, with metabolic regulation predominantly driven by the uncarboxylated isoform of it [16, 17]. The inability to analyze these isoforms separately is a recognized limitation of this study. Moreover, the serum level of a biomarker of osteoblast activity, e.g., B-ALP, was found to be lower compared to that of healthy pregnant women. While glucose level is not regulated by B-ALP levels, a reduced level of it may suggest compromised bone mineral density in GDM patients, which is potentially linked to its low level of circulatory level [18]. Studies have shown that dysfunctional insulin metabolism in type 2 diabetes is associated with lower bone formation [19], and equally, a comparable mechanism may also be relevant in the context of GDM. The lack of significant variation in the level of CTX suggests that variability in glucose level during GDM may primarily influence osteoblast function, with minimal effects on osteoclast, and so warrants further investigations on this aspect. Overall, our findings align with previous observations where Gong *et al.* reported a persistent reduced level of osteocalcin in women with GDM after delivery, and these women were found to be at higher risk of abnormal glucose metabolism [11]. Similarly, Hwang *et al.* have found that pregnant women with higher osteocalcin concentrations demonstrated greater insulin sensitivity [20]. Collectively, the evidence underscores a bone-pancreas interaction, with done-derived factors contributing to the control of glucose regulation and energy metabolism. Assessment of bone turnover markers alongside metabolic parameters may help identify women at higher risk of GDM, characterized by insulin resistance, elevated BMI, and low osteocalcin levels.

Although the relationship between gestational diabetes mellitus and bone turnover is illuminated, due to the cross-sectional study design, it is impossible to draw conclusions based on the causal or time relationship. Moreover, the inability to differentiate uncarboxylated and carboxylated osteocalcin, which play different functions in metabolism, and the failure to directly determine the bone mineral density are significant drawbacks of the methods. Longitudinal and mechanistic research on bone-derived factors and glucose metabolism in and after pregnancy requires the inclusion of analyses of osteocalcin isoforms and follow-ups in the postpartum period to enhance understanding of the relation between bone-derived factors and glucose metabolism.

CONCLUSIONS

The research revealed that there is a close relationship between GDM and bone turnover markers. The findings of the research showed that GDM is associated with lower bone turnover marker levels, particularly osteocalcin. Osteocalcin, as well as BMI and HOMA-IR, had independent relationships with GDM, and the role of the bone metabolism biomarker in affected women is possible. Although the study demonstrates the association, it does not allow making causal or predictive inferences because the study is cross-sectional. More studies should be

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conducted to affirm these results, as well as to investigate the biological processes that are involved, e.g. the interaction between the bone and pancreas.

Authors Contribution

Conceptualization: WUH, MA, MM, WA, FA, AA
Methodology: MA, IA, WA, FA, MNM,
Formal analysis: WUH, MM, WA, ZMK
Writing and Drafting: MM, IU, WA, FA, AA, MA
Review and Editing: WUH, MA, MM, IU, WA, FA, MNM, AA, MA, ZMK

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

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