Cord Blood Leptin, Osteocalcin, and Tumor Necrosis Factor Alpha Levels in Gestational Diabetic Versus Normal Pregnancy: Impact on Neonatal Outcome in a Birth Cohort

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Abstract

Background: Although gestational diabetes may result in elevated leptin, osteocalcin, and TNF- α (Tumor necrosis factor) levels, impact of those parameters on the neonatal outcome is not well established.

Objectives: The aim of the current study was to evaluate whether cord blood leptin, TNF α (Tumor necrosis factor), and osteocalcin have a predictive value for neonatal complications due to maternal GDM (Gestational Diabetes Mellitus).

Methods: This cohort study was performed at Near East University Hospital, Nicosia, Cyprus. All pregnant females under follow-up of the department of Obstetrics and Gynecology between years 2010 and 2012 were invited to participate. Those, who gave consent, were enrolled. Expecting mothers were categorized as GDM and normal pregnancy. 41 (21.5%) of the pregnancies were GDM and 159 (78.5%) were normal. Cord blood samples were obtained at delivery. Osteocalcin, leptin, and TNF- α levels and neonatal complications were evaluated thereafter.

Results: Comparison of pregnancy complications between groups revealed no significant differences except for more frequent maternal hypothyroidism in the GDM group ($P < 0.001$). The GDM group's cord blood osteocalcin levels (44.69 ± 27.6 ng/mL and 35.23 ± 22.9 ng/mL in the control group ($P : 0.037$)) and frequency of neonatal hypoglycemia were significantly higher than controls ($P : 0.047$), whereas cord blood TNF α and leptin levels were not statistically different ($P > 0.05$). Newborns with hypoglycemia showed higher cord blood osteocalcin, TNF- α , and leptin. In addition, osteocalcin levels were significantly higher in cord blood of LGA babies born from GDM mothers (50.41 ± 32.79 and 35.19 ± 20.18 ng/mL).

Conclusions: In this study, cord blood leptin, osteocalcin, and TNF- α were significantly correlated with neonatal hypoglycemia. Moreover, cord blood osteocalcin levels were significantly higher in the GDM group. This increase in osteocalcin in the GDM group, may be related to the regulation of blood glucose levels by osteocalcin.

Keywords: Cord Blood, Gestational Diabetes, Hypoglycemia, Leptin, Osteocalcin, Tumor Necrosis Factor-alpha

1. Background

Gestational diabetes mellitus (GDM) is defined as glucose intolerance, first recognized during pregnancy, with a prevalence of 5% to 7% in different populations (1). The complications of GDM depend on the mean glucose level and the duration of hyperglycemia during pregnancy. This means that longer duration and amplitude of hyperglycemia in the fetus leads to more complications. Females, who have GDM, have a higher incidence of Type 2 diabetes mellitus and obesity in the next ten years and the incidence of hypertension and urinary tract infections are

more common during pregnancy (1).

Numerous studies have reported that high maternal blood glucose is associated with fetal morbidity, macrosomia, and subsequent complications, such as hypoglycemia, polycythemia, respiratory distress, cardiac anomalies, sacral dysgenesis, hyperbilirubinemia, birth trauma, perinatal asphyxia, intrauterine growth retardation, hypocalcemia, and hypomagnesemia in the neonatal period (1, 2).

Obesity and GDM cause high inflammatory cytokine production with chronic and low-grade inflammation.

Previous studies reported a correlation between GDM and early pregnancy blood leptin, adiponectin, and TNF- α (2-4). In addition, some clinical studies have shown increased umbilical cord leptin and TNF- α in GDM. In addition, serum leptin levels and insulin resistance was shown to have a positive correlation. Liu et al. demonstrated that first-trimester serum TNF- α levels might be a useful marker to predict for GDM (2-4). In addition, skeleton-derived osteocalcin has been shown to have significant effects on beta cell proliferation of pancreas, increasing insulin sensitivity, secretion, and glucose tolerance (5, 6). Some studies reported higher GDM while others reported normal levels. The relationship between GDM and osteocalcin was not certain (7-12).

In this study, cord blood leptin, TNF- α , and osteocalcin levels were measured during labor in pregnant females, with and without GDM, in order to evaluate whether these parameters predicted neonatal complications due to GDM.

2. Methods

2.1. Subjects and Methods

Ethics Statement: Informed parental consent was obtained from each participant, and the study protocol was approved by Near East University, Nicosia, North Cyprus Ethics Committee (No:2011/35), according to the declaration of Helsinki (2008).

2.2. Participants

All pregnant females followed by the department of Obstetrics and Gynecology of Near East University Hospital, Nicosia, North Cyprus, between September 2010 and April 2012, were invited to participate in this cohort study (Figure 1). Written informed consent for obtaining a cord blood sample from expecting mothers were obtained. Those, who accepted to participate, were enrolled in the study. After enrollment, the newborn's umbilical cord blood was collected following labor in the delivery room and the newborn were followed postnatally by the same neonatology at the department of pediatrics of NEU Hospital, which is the only referral university hospital in North Cyprus. Exclusion criteria included congenital anomalies (cardiac, pulmonary, gastrointestinal, and cranial complications), metabolic diseases, TORCH infections, and not giving consent to participate.

The diagnosis of GDM was made using the American Diabetic Association's recommendations. Oral glucose tolerance test (OGTT) with 75 gr of oral glucose was performed between the 24th and 28th gestational weeks. Pregnant females were diagnosed as GDM when the following values were higher than cut off (fasting glucose > 95 mg/dL, 1st

hour > 180 mg/dL, and 2nd hour > 155 mg/dL). The participants with GDM were consulted at the Division of Endocrinology of the researcher's hospital and they were offered a diet or insulin if the diet was not sufficient to regulate blood glucose levels. Inclusion criteria were being a healthy pregnant female and the presence of GDM. The participants diagnosed as GDM were included in the GDM group, while those with normal OGTT made up the control group. All participants' pregnancy history was taken (early membrane rupture, preeclampsia, eclampsia, uteroplacental insufficiency, hypothyroidism, urinary tract infection, anemia, and vaginitis) at the time of delivery by the attending pediatrician. The pregnancy history results are shown in Table 1.

Anthropometric measurements: the variables of the neonates included birth weight, length, head circumference, gestational age (GA), and ponderal index (PI). The clinical characteristics and anthropometric measurements are shown in Table 1. PI was calculated as body weight (kg)/body length (m³) and normal range was accepted at 2.32 to 2.85, as recommended. The body weight of each neonate and placental weight were determined to the nearest 1 g using an electronic scale. Body length was determined to the nearest 0.1 cm in the supine position with a length board. Head circumference was determined with a plastic tape to the nearest 0.1 cm.

Sample collection and biochemical analysis: five milliliters of cord blood samples were obtained from all newborns. Each blood sample was left to coagulate for 30 minutes, then centrifuged at 1000 g for 15 minutes to separate serum. Serum aliquots were immediately labeled and stored at -80°C until analysis.

Serum leptin and TNF- α were measured by the Solid Phase Enzyme Amplified Sensitivity Immunoassay (EIA) method (DIAsource Immunoassay S.A., Belgium), and osteocalcin was measured by the Electrochemiluminescence Immunoassay method on Roche Cobas e601 auto-analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The methods of measurements were carried out according to the manufacturer's instructions by an investigator blinded to the clinical data.

The standard curves for Leptin and TNF- α were created by reducing the data using a computer software (Softmax Pro.) capable of generating four parameter logistic (4PL) curve fit. The lower detection limits were 0.04 ng/mL for Leptin, 0.7 pg/mL for TNF- α , and 0.5 ng/mL for osteocalcin.

Postnatal follow-up of neonatal complications of GDM: All newborns were followed for hypoglycemia, jaundice, hypocalcemia, hypomagnesemia, asphyxia, and polycythemia. Hypoglycemia was diagnosed if the capillary blood glucose level was less than 45 mg/dL. Physiologic jaundice was diagnosed if jaundice started after the first

*Osteocalcin, TNF α , Leptin

Figure 1. Study Design and Flow (GDM : Gestational Diabetes Mellitus)

24 hours of life and if serum bilirubin level was more than 12 to 13 mg/dL in term and 15 mg/dL in preterm infants, if daily bilirubin increasing rate was more than 2 mg/dL, and if jaundice lasted one week in term and two weeks in preterm infants. Hypocalcemia was diagnosed if the serum calcium level was less than 7 mg/dL. Hypomagnesemia was diagnosed if the serum magnesium level was less than 1.5 mg/dL. Blood glucose levels were checked at the 1st, 2nd and the 6th hour and venous hematocrit was checked in the 6th hour of life in all newborns, consisting of the GDM group.

Gestational weeks were calculated by the last due date and if necessary Ballard score was used. The diagnosis of small for gestational age (SGA), average for gestational age (AGA) and large for gestational age (LGA), was given using normal ranges of Turkish children. Perinatal asphyxia was diagnosed, if cord blood gas Ph was less than 7.1 and if APGAR score was less than six during the 1st or 5th minute of life.

Statistical analysis and sample size: Before the start of the study, a power analysis was applied to calculate the required sample size to achieve 80% statistical power with a

confidence interval of 95% and 5% level of significance. Results showed that a sample of 147 patients would be sufficient to reach the goal.

All statistical analyzes were performed using IBM SPSS version 22.0, 2013 for Windows (IBM Corp., Armonk, N.Y., USA). The results were expressed as mean $\pm$ standard deviation (SD). Groups were compared using the Student's t-test for normally distributed data. To determine the relationship between principal variables and the other continuous variables, Pearson correlation or Spearman non-parametric correlation tests were used. When equality of variances was not present, the Kruskal Wallis and Mann-Whitney U nonparametric tests were used. P values of $P < 0.05$ were considered statistically significant for all analyses.

3. Results

Among all pregnant females under follow-up (n:250) by the Department of Obstetrics and Gynecology, 220 of which accepted to participate in the study following informed consent. Thirty pregnant females were excluded

Table 1. Comparison of Pregnancy History, Neonatal Demographic Characteristics, and Postnatal Problems^a

	GDM + Group	Control	P Value
Pregnancy History			
Early Membrane rupture	2 (5)	4 (2)	0.910
Preeclampsia	0	1 (0.7)	0.567
Eclampsia	0	1 (0.7)	0.596
Uteroplacental insufficiency	0	1 (0.7)	0.596
Hypothyroidism	16 (39)	17 (11)	0.001 ^b
Urinary tract infection	11 (27)	26 (17)	0.209
Anemia	3 (7)	7 (5)	0.537
Vaginitis	3 (7)	11 (7)	0.951
Early delivery risk	2 (5)	4 (2)	0.514
Infertility	3 (7)	3 (2)	0.097
Polyhydramnios	2 (5)	4 (3)	0.502
Oligohydramnios	0	5 (3.3)	0.228
Hypertension	0	1 (0.7)	0.06
Newborns Demographic Characteristics			
Weight, g	3311 ± 232	3455 ± 412	0.409
Height, cm	49.17 ± 2.15	48.57 ± 1.9	0.07
Head circumference, cm	35.28 ± 1.59	35.42 ± 1.28	0.454
Gestational weeks, w	37.25 ± 6.1	38.18 ± 3.3	0.201
Ponderal index	2.79 ± 0.33	2.83 ± 0.32	0.564
Caesarean section/Normal spontaneous delivery	32/6	118/15	0.4
AGA	25 (61)	107 (71)	0.406
LGA	13 (32)	34 (23)	
SGA	3 (7)	9 (6)	
Boy/girl (n/n)	18/23	77/73	0.4
Postnatal Problems			
Internation to NICU	5 (12)	5 (3)	0.06
Respiratory Distress	3 (7)	7 (5)	0.163
Hypoglycemia	1 (2.4)	1 (0.7)	0.047 ^b
Neonatal Jaundice	1 (2.4)	7 (5)	0.221
Asphyxia	0	0	
Symptomatic hypocalcemia	0	0	
Symptomatic hypomagnesemia	0	0	

Abbreviations: AGA, average gestational age; GDM, gestational diabetes mellitus; LGA, large for gestational age; NICU, neonatal intensive care unit; TNF, Tumor necrosis factor; SGA, Small for gestational age.

^aValues are expressed as mean ± SD or No. (%).

^bStatistically significant (Chi-square test).

based on the exclusion criteria. Finally, 191 pregnant females were enrolled in the study. Of those, 41 were diag-

nosed as GDM and 159 as healthy pregnancies (controls). Newborns were also categorized as the GDM group based on the GDM history of their mother and control.

Pregnancy histories, demographic characteristics, and postnatal problems of newborns are presented in [Table 1](#). There was no statistically significant difference based on pregnancy history between the two groups of mothers except for the higher percentage of hypothyroidism in the GDM group ($P : 0.001$) ([Table 1](#)). In addition, no significant differences were detected, in comparison of birth weight, birth height, head circumference, PI, type of delivery, and gender between groups ($P > 0.005$).

Comparison of the two groups of newborns based on respiratory distress, neonatal jaundice, hypomagnesemia, hypocalcemia, and perinatal asphyxia revealed no statistically significant differences, while, hypoglycemia was more common in the GDM newborn group ($P : 0.002$). Although there was no statistically significant difference in the frequency of NICU admissions between groups, it was more frequent in the GDM newborn group ($P : 0.06$) ([Table 1](#)).

Cord blood osteocalcin, TNF- α and leptin levels between groups are presented in [Figure 2](#). Although cord blood osteocalcin levels (mean $\pm$ SD of 44.69 ± 27.6 ng/mL, and 35.23 ± 22.9 ng/mL, respectively) were significantly higher in the GDM group ($P : 0.037$), cord blood TNF- α (mean $\pm$ SD of 10.16 ± 2.65 pg/mL in the GDM group and 10.01 ± 3.42 pg/mL in the control group; $P : 0.813$) and leptin values (2.70 ± 2.3 mg/mL in GDM group and 3.40 ± 2.5 ng/mL in control group ($P : 0.212$)) were not significantly different.

When newborns were compared according to gender, there was no significant difference between the mean weight of different genders ($P : 0.255$). Mean cord serum osteocalcin value was higher in males (38.98 ± 2.65 ng/mL in males and 35.36 ± 2.53 ng/mL in females) demonstrating no significant difference ($P : 0.327$). Comparison of cord blood leptin and TNF α levels based on gender revealed no statistical differences between groups.

Cord blood levels of leptin, TNF- α , and osteocalcin of newborns < 4000 g vs. ≥ 4000 g were compared demonstrating no significant difference in mean leptin and TNF α values ([Table 2](#)). Osteocalcin levels of newborns weighing ≥ 4000 g was significantly higher than those weighing ≤ 4000 g.

Comparison of cord blood TNF α , leptin, and osteocalcin values of the GDM group based on the presence of AGA, LGA, and SGA demonstrated no significant differences between groups ([Table 3](#)). Only the LGA subgroups' mean osteocalcin level was higher ([Table 3](#)).

On the other hand, osteocalcin levels of the GDM LGA newborn subgroup (mean $50,41 \pm 32,79$ ng/mL) was signif-

Figure 2. Comparison of Cord Blood Osteocalcin, TNF α and Leptin Levels of GDM and Control Groups

(GDM: gestational diabetes mellitus group). Cord blood osteocalcin levels of the GDM group were significantly higher compared to the control group. ($P: 0.037$, Mann-Whitney U test).

Table 2. Comparison of Cord Blood Osteocalcin, TNF- α and Leptin Levels of Newborns Weighing < 4000 g Versus $\geq$ 4000 g

	Weight, g	N	Mean $\pm$ SD	P Value
Osteocalcin, ng/mL	> 4000	41	36,012 $\pm$ 18.11	0.049 ^a
	< 4000	133	37.56 $\pm$ 25.86	
TNF α, pg/mL	> 4000	39	9.78 $\pm$ 2.40	0.616
	< 4000	116	10.13 $\pm$ 3.53	
Leptin, ng/mL	> 4000	34	3.25 $\pm$ 2.37	0.774
	< 4000	95	3.27 $\pm$ 2.55	

^aStatistically significant (student t test).

icantly higher when compared with the control newborn LGA subgroup (35.19 ± 20.18 ng/ ($P: 0.047$), while cord TNF- α and leptin mean levels of GDM LGA and control LGA subgroups revealed no significant differences ($P > 0.05$) (Table 4 and Figure 3).

Finally, when newborns were compared based on the presence of neonatal hypoglycemia, cord blood osteocalcin, leptin, and TNF α were significantly higher in those, who developed neonatal hypoglycemia ($P < 0.05$).

Table 3. Comparison of Cord Blood Osteocalcin, TNF α and Leptin Levels of LGA, AGA and SGA Born Newborns from GDM Mothers

		N	Mean $\pm$ SD	P Value
Osteocalcin, ng/mL	AGA	22	41,05 $\pm$ 23,88	0,474
	LGA	12	52,68 $\pm$ 34,78	
	SGA	2	36,80 $\pm$ 15,61	
	Total	36	44,69 $\pm$ 27,62	
TNF α, pg/mL	AGA	19	10,15 $\pm$ 2,81	0,537
	LGA	11	10,46 $\pm$ 2,44	
	SGA	1	7,3020	
	Total	31	10,17 $\pm$ 2,65	
Leptin, ng/mL	AGA	15	2,20 $\pm$ 1,42	0,002 ^a
	LGA	9	2,73 $\pm$ 2,34	
	SGA	1	10,00	
	Total	25	2,70 $\pm$ 2,32	

Abbreviations: AGA, average gestational age; GDM, gestational diabetes mellitus; LGA, large for gestational age; SGA, small for gestational age; TNF, tumor necrosis factor.

^aStatistically significant. Leptin level was very high in the only SGA newborn compared to others.

4. Discussion

In the current study, newborns born from mothers with and without GDM, demonstrated no statistically sig-

Figure 3. Comparison of Cord Blood Osteocalcin, TNF α and Leptin Levels of GDM-LGA and Control-LGA Subgroups

(GDM: gestational diabetes mellitus group, LGA: large for gestational age). Cord blood osteocalcin levels of the LGA newborns born from GDM mothers were statistically significantly higher than newborns born from healthy mothers ($P: 0.047$, Mann Whitney U test).

Table 4. Comparison of Cord Blood Osteocalcin, TNF α and Leptin Levels of GDM LGA and Control LGA Subgroups

	GDM	N	Mean $\pm$ SD	P Value
Osteocalcin, ng/mL	-	39	35,19 $\pm$ 20,18	0.047 ^a
	+	14	50,41 $\pm$ 32,79	
TNF α , pg/mL	-	36	9,47 $\pm$ 2,19	0.34
	+	12	10,19 $\pm$ 2,50	
Leptin, ng/mL	-	33	3,46 $\pm$ 2,21	0.997
	+	10	3,46 $\pm$ 3,19	

Abbreviations: GDM, gestational diabetes mellitus; LGA, large for gestational age; TNF, tumor necrosis factor.

^aStatistically significant.

nificant differences based on anthropometric measurements, weight, height, head circumferences, and PI. Only LGA newborns were more common in those with a GDM mothers, as expected. Moreover, the frequency of postnatal problems of newborns, such as macrosomia, asphyxia, hypocalcemia, hypomagnesemia, newborn jaundice, polycythemia, and respiratory distress were not statistically different, yet hypoglycemia was more common in the GDM group, as predictable.

There was no difference between cord blood TNF- α and

leptin levels, while, osteocalcin was higher in the GDM group. Comparison of males and females based on cord blood leptin, osteocalcin, and TNF α levels revealed no significant differences. In addition, cord blood osteocalcin and TNF α levels of LGA and SGA newborns did not show any significant differences, whereas leptin was higher in the only SGA baby.

In addition, osteocalcin, leptin, and TNF- α were higher in those with neonatal hypoglycemia compared to normoglycemic neonates.

According to the results of recent studies, the incidence of abnormal glucose metabolism was 3% to 8% in different populations. The most common reason for glucose metabolism abnormality was GDM (80%) during pregnancy. Although glucose intolerance is mild, it has fetal and neonatal complications exerted by hyperglycemia. In the current study, all pregnant GDM participants were under regular follow-up by the endocrinology clinic of the University Hospital, which may be the main reason for detecting fewer side effects due to hyperglycemia.

Recent studies showed that blood leptin, adiponectin, and TNF- α in early pregnancy were correlated with GDM. Furthermore, TNF- α and Interleukin-6 (IL-6) levels were more increased in placenta-derived tissue cultures in GDM when compared to normal controls (13-15). Moreover,

blocking TNF- α signaling mechanism increases insulin sensitivity (4). It has been postulated that cytokines, especially TNF α , IL-6, and leptin play essential roles in metabolic irregularity during pregnancy, which may trigger GDM (13-15). Moreover, TNF- α is an important predictor of insulin resistance during late pregnancy. In the current study, although there was no difference between GDM and control groups, based on the studied parameters, when newborns with and without neonatal hypoglycemia were compared, osteocalcin, TNF- α , and leptin levels were significantly higher in the hypoglycemic group.

Leptin is higher in preeclamptic pregnancies and pregnant females being treated with insulin. It also plays an important role in GDM, intrauterine growth retardation, preeclampsia, and birth weight (16, 17). Some studies that evaluated the effect of maternal leptin levels on birth weight and adiposity of newborns, reported no association between maternal leptin and adiposity of newborns (18), whereas others reported a positive correlation (19). These differences may be related to different sampling times. The current study is different from previous reports, in that the sampling was obtained from cord blood, and not during pregnancy from the mother as done previously.

Several studies reported the difference in leptin levels between genders (19-23). Males from diabetic mothers demonstrated higher leptin levels in the study of Jahan et al. (23). On the other hand, leptin levels of healthy female newborns were higher in the study of Mouzaki et al. (24) and Shih-Ping et al. (25). Cord blood leptin levels of female and male newborns were not different within both the GDM and normal groups in the current study. Meanwhile, Mouzaki et al. reported higher cord leptin levels in LGA newborns, while cord leptin level was extremely high in the SGA newborns (26).

In the current study, the comparison of GDM and normal pregnancies revealed no differences in pregnancy complications and neonatal problems, except for maternal hypothyroidism and neonatal hypoglycemia being more common in the GDM group ($P : 0.047$). Thyroxine and insulin have essential effects on each other's endocrine activity. Recent studies showed that hypothyroidism increases insulin resistance (25, 27, 28). Coexistence of hypothyroidism and insulin resistance supports this correlation. On the other hand, some authors suggest that this correlation depends on age, gender, weight, race, and number of pregnancies. According to the study of Cleary-Goldman et al. (29), which included 10,000 pregnant females, no correlation between GDM and hypothyroidism was detected. In addition, D'Amelio et al. (27) found that thyroid antibody-positive pregnant females were at a higher risk of developing diabetes in their future life. In the current study, hypothyroidism was more common in

the GDM group compared to controls ($P : 0.001$). Further studies, to explain the underlying pathophysiology of this correlation, are warranted.

Skeleton does not only involve the bones, and also acts as an endocrine organ, by stimulating beta cell proliferation of pancreas and insulin sensitivity, especially, secretion and glucose tolerance by osteocalcin (28). Uncarboxylated form of osteocalcin, exerts effects on energy and glucose metabolism (30). Some studies have reported higher cord blood osteocalcin levels in GDM, (7-9), while others (10) demonstrated lower osteocalcin levels in GDM compared to normal pregnancies. In addition, studies comparing 2nd and 3rd-trimester cord blood osteocalcin levels of GDM females with controls demonstrated higher levels in GDM pregnant females (9, 11). On the other hand, serum osteocalcin levels were found to be lower in type 2 diabetics in some studies (12). In accordance with most studies, in the current study osteocalcin levels were higher in the cord blood of GDM pregnant females.

The relatively low sample size and the participants that were enrolled from one center, were the main limitations of the current study. A higher number of LGA newborns would improve the power of the study. On the other hand, this is the first cohort study, comparing the impact of cord blood osteocalcin, leptin, and TNF- α on neonatal outcome of GDM and control pregnancies.

In addition, regulation of blood glucose during pregnancy of all GDM females was followed carefully by the same endocrinologist in the university hospital of the current study. This close monitorization may be the reason for low neonatal complications. It would be better if HbA1c levels were studied to confirm the well-controlled blood glucose levels during pregnancy.

This study demonstrated that higher cord blood leptin, osteocalcin, and TNF- α are related to the development of neonatal hypoglycemia. Furthermore, GDM demonstrated higher levels of cord blood osteocalcin levels. These results suggest that follow-up of osteocalcin levels during pregnancy in GDM may predict neonatal hypoglycemia. Further studies are warranted to delineate this issue.

Footnote

Conflict of Interest: We do not have any conflict of interest.

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