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The Role of Early Pregnancy Maternal pGCD59 Levels in Predicting Neonatal Hypoglycemia—Subanalysis of the DALI Study

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Abstract

Context

Neonatal hypoglycaemia (NH) is the most common metabolic problem in infants born of mothers with gestational diabetes. Plasma glycated CD59 (pGCD59) is an emerging biomarker that has shown potential in identifying women at risk of developing gestational diabetes. The aim of this study was to assess the association between early maternal levels of pGCD59 and NH.

Objective

The aim of this study was to assess the association between early pregnancy maternal levels of plasma glycated CD59 (pGCD59) and neonatal hypoglycemia (NH).

Methods

This is an observational study of pregnant women with a prepregnancy body mass index (BMI) greater than or equal to 29 screened for eligibility to participate in the Vitamin D and Lifestyle Intervention for Gestational Diabetes (DALI) trial. This analysis included 399 pregnancies. Levels of pGCD59 were measured in fasting maternal samples taken at the time of a 75-g, 2-hour oral glucose tolerance test performed in early pregnancy (< 20 weeks). NH, the study outcome, was defined as a heel-prick capillary glucose level of less than 2.6 mmol/L within 48 hours of delivery.

Results

We identified 30 infants with NH. Maternal levels of pGCD59 in early pregnancy were positively associated with the prevalence of NH (one-way analysis of variance, $P < .001$). The odds of NH were higher in infants from mothers in tertile 3 of pGCD59 levels compared to those from mothers in tertile 1 (odds ratio [OR]: 2.41; 95% CI, 1.03-5.63). However, this was attenuated when adjusted for maternal BMI (OR: 2.28; 95% CI, 0.96-5.43). The cross-validated area under the curve (AUC) was 0.64 (95% CI, 0.54-0.74), and adjusted for maternal BMI, age, and ethnicity, the AUC was 0.70 (95% CI, 0.56-0.78).

Conclusion

Although pGCD59 levels in early pregnancy in women with BMI greater than or equal to 29 are associated with NH, our results indicate that this biomarker by itself is only a fair predictor of NH.

Keywords: neonatal hypoglycemia, glycation, biomarkers, prediction, epidemiology

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Women with gestational diabetes mellitus (GDM), especially those developing GDM early in pregnancy, are at higher risk of maternal and fetal adverse outcomes (1). Plasma glycated CD59 (pGCD59), an emerging diabetes biomarker (2, 3), reportedly exhibits high sensitivity and specificity for the diagnosis of GDM not only at 24 to 28 gestational weeks, the current standard of care time for screening/diagnosis of GDM, but also early in pregnancy (< 20 weeks of gestation) (4-6). CD59 is a glycoprotein universally expressed in mammalian cells (7) whose role it is to inhibit formation of the complement membrane attack complex protecting cells from complement-mediated damage (8). In diabetes, CD59 becomes inactivated through nonenzymatic glycation of its Lys41 amino acid residue forming glycated CD59 (GCD59) (9). A soluble form of GCD59 shed from cell membranes can be measured with a highly sensitive and specific enzyme-linked immunosorbent assay (ELISA) test (2). Zhao et al (10) found that down-regulation of CD59 predisposed the placenta to increased complement activation and complement-mediated damage as compared to normal pregnancy. Potentially this can lead to placental dysfunction and in turn to a decrease in nutrient circulation to the fetus, intrauterine growth restriction, reactive increase in catecholamines, and decreased insulin secretion ante partum with compensatory increased insulin secretion post partum leading to hypoglycemia (11).

Neonatal hypoglycemia (NH), defined as a capillary glucose level of less than 2.6 mmol/L, is the most common metabolic problem in offspring born to women with GDM (12, 13). In maternal hyperglycemic states, the free passage of glucose through the placenta leads to elevated glucose levels in the fetus that, in turn, leads to fetal hyperinsulinemia (14). Post partum, as the maternal-fetal glucose transport stops, the high neonatal insulin levels may cause infant hypoglycemia. Even in normoglycemic mothers, fetal hyperinsulinemia will lower infant glucose concentrations by increasing glucose clearance into the tissues (14). There is evidence that maternal hyperglycemia–induced fetal hyperinsulinemia could begin as early as the first trimester of pregnancy (15). NH can cause brain injury (16), and the potential long-term consequences of this condition make it a significant cause of newborn admission to intensive care units (17). A systematic review and meta-analysis (17) that included 1675 infants found that NH was associated with a 3-fold increased risk in visual-motor impairment and executive dysfunction at age 4 years. NH was linked to a more than 3-fold increased risk of neurodevelopmental impairment in older children aged 6 to 11 years, as well as a 2-fold increased risk of inadequate numeracy

and literacy (17). Identification of pregnancies at risk of NH would allow close monitoring both of mother and offspring and early intervention that could potentially reduce short-term and long-term infant adverse outcomes.

We recently reported that in samples from participants in the pan-European Vitamin D and Lifestyle Intervention (DALI) study (18), plasma levels of GCD59 were associated with a diagnosis of GDM in early pregnancy (< 20 weeks' gestation) and were positively associated with the prevalence of large for gestational age (LGA) newborns (5) in women with a body mass index (BMI) greater than or equal to 29. We aimed to study the association between early pregnancy (< 20 weeks' gestation) pGCD59 and the presence of NH in infants of women at high risk of GDM.

Materials and Methods

Type of Study and Design

We conducted an ancillary study addressing the relationship between pGCD59 in frozen plasma samples collected from individuals screened for eligibility to participate in the DALI study, a multicenter, randomized controlled trial conducted across 9 European countries (18, 19), and NH.

Participants and Selection of Cases and Controls

A total of 1046 consecutive pregnant women of gestational age between 8 and 19 + 6 weeks (hereafter termed *< 20*), aged 18 years or older, with a singleton pregnancy and with a prepregnancy BMI of greater than or equal to 29, were screened for eligibility to enroll in the DALI study. All participants underwent a 75-g, 2-hour oral glucose tolerance test (OGTT) at less than 20 weeks. Plasma samples used for pGCD59 analysis were drawn simultaneously with the fasting sample of the OGTT conducted at less than 20 weeks of gestation. Women who did not meet the International Association of Diabetes and Pregnancy Study Group (20) criteria for GDM (fasting value ≥ 92 mg/dL [5.1 mmol/L], 1-hour value ≥ 180 mg/dL [10 mmol/L], and 2-hour value ≥ 153 mg/dL [8.5 mmol/L]) were enrolled in the DALI study. Of the 1046 women screened for eligibility at less than 20 weeks of gestation, we included 399 pregnancies for which information regarding neonatal glycemic status was available.

Study Variables

Maternal age in years, ethnicity, BMI at the first prenatal visit, and neonatal characteristics (birth weight, birth weight percentile categorized as LGA, small for gestational age [SGA], appropriate for gestational age [AGA], and prematurity defined as gestational age at birth < 37 weeks) were included. LGA was defined as infant birth weight greater than the 90th percentile for their gestational age and sex; SGA was defined as infant birth weight below the 10th percentile for babies of the same gestational age and sex. Maternal ethnicity was categorized as White and Other. The primary outcome was the diagnosis of NH defined as a capillary glucose level of less than 2.6 mmol/L (47 mg/dL) drawn from a heel prick within 48 hours of delivery (18). pGCD59 was measured in deidentified, coded plasma samples collected in the fasting state using the ELISA assay (RRID: AB_2921233) that reports pGCD59 levels in stan-

dard peptide units (SPU), as described previously (2). Test operators were blinded to the participants' demographic or clinical information. The interassay coefficient of variation was less than 10.0%. Maternal levels of pGCD59 in SPU were the main predictor variable. Additional variables included were glycated hemoglobin A_{1c} (HbA_{1c}), GDM status (< 20 weeks), and umbilical cord C-peptide levels.

Statistical Analysis

Maternal sociodemographic and anthropometric characteristics and neonatal characteristics were described according to the NH status using counts and proportions. To describe pGCD59 distribution across levels of the study covariates we used means and SDs. We used tertile levels for pGCD59 analysis to contrast the highest vs the lowest tails of the distribution of pGCD59. The difference in mean pGCD59 according to NH status, maternal and neonatal sociodemographic, and anthropometric characteristics were computed. To assess the evidence of different mean pGCD59 values by NH status and/or other variables, we used robust analyses of variance.

The association between pGCD59 and the presence of NH was assessed using logistic regression models with NH as the binary outcome. We fitted 4 different models adding, at each time, 1 additional covariate to control for confounding. Model 4 includes as independent variables the tertiles of pGCD59, maternal BMI, age, and ethnicity. The goodness of fit of the models was assessed using the Hosmer-Lemeshow test (21). We derived conditional odds ratios (ORs) and their 95% CIs. We developed similar models for HbA_{1c}, fasting plasma glucose (FPG), and GDM status (< 20 weeks' gestation). We selected GDM status as the testing variable and not individual OGTT glucose values results because it encompasses the totality of positive cases and increases the power of the analysis. The correlation between pGCD59 levels and cord C-peptide levels was assessed by Pearson correlation (Pearson correlation coefficient: R).

Finally, the accuracy of the final model was assessed using receiver operating characteristic (ROC) curves, adjusted for maternal age, BMI, and ethnicity. The area under the curve (AUC) was derived using the DeLong, DeLong, and Clarke-Pearson nonparametric tied corrected estimator (22). Afterward, under nonparametric estimation, SEs and AUC 95% CIs were derived using cross-validation and bootstrapping procedures with 1000 replications. All analyses were performed using Stata v.16.1 statistical software (StataCorp).

Partners Healthcare institutional review committees approved the protocol for this study (Protocol: 2011P002254/BWH). The DALI trial was registered as a randomized controlled trial November 21, 2011 (ISRCTN70595832).

Results

Table 1 summarizes maternal characteristics and neonatal outcomes by NH status. Mothers of infants with NH had statistically significantly higher levels of pGCD59 (3.2 vs 2.7 SPU; $P = .004$) and tertile 3 of maternal pGCD59 showed the highest proportion of NH (14.6% vs 4.2% and 6.6% for tertile 2 and tertile 1, respectively; $P = .012$). HbA_{1c} was statistically significantly higher in mothers of infants with NH (5.4 vs 5.2% [36 vs 33 mmol/mol]; $P = .015$); however, there was not a statistically significant difference in the proportion of HbA_{1c} tertiles. OGTT glucose values were statistically significantly higher in the NH subgroup at all 3 time points ($P <$

.001), and a positive GDM status (< 20 weeks of gestation) was associated with the development of NH ($P < .001$). There was no statistically significant difference between women whose infant developed NH and those who did not in terms of age or ethnicity; however, maternal BMI was statistically significantly higher in the NH subgroup ($P = .001$), a BMI greater than or equal to 35 being associated with a higher rate of NH ($P = .001$).

Table 1.

Maternal and infant characteristics by neonatal hypoglycemia status less than 20 gestational weeks (n = 399)

	Neonatal hypoglycemia		
	No	**Yes**	
	n = 369	**n = 30**	
Maternal variables	**n (%) Mean (SD)**	**n (%) Mean (SD)**	***P*[a]**
Maternal pGCD59, SPU	2.7 (0.8)	3.2 (1.3)	.004
Maternal pGCD59, SPU			.012
Tertile 1 (≤ 2.5)	155 (93.4%)	11 (6.6%)	
Tertile 2 (> 2.5 ≤ 3.2)	137 (95.8%)	6 (4.2%)	
Tertile 3 (> 3.2)	76 (85.4%)	13 (14.6%)	
Maternal HbA_{1c}, % (mmol/mol [b])	5.2 (0.4) (33 (2.5))	5.4 (0.4) (36 (2.6))	.015
Maternal HbA_{1c}, % (mmol/mol [b])			.164
Tertile 1 (≤ 5.1 (32))	141 (95.9%)	6 (4.1%)	
Tertile 2 (> 5.1 (32) ≤ 5.5 (37))	93 (94.9%)	5 (5.1%)	
Tertile 3 (> 5.5 (37))	98 (89.9%)	11 (10.1%)	
Maternal OGTT, mmol/L			
Fasting	4.6 (0.4)	4.9 (0.7)	< .001
1 h	6.8 (1.6)	8.4 (2.6)	< .001
2 h	5.8 (1.2)	7.1 (2.0)	< .001
Maternal GDM status			< .001
No	322 (95.8%)	14 (4.2%)	
Yes	47 (75.8%)	15 (24.2%)	
Maternal age, y	32.8 (5.3)	32.3 (4.8)	.591
Maternal age in categories, y			.196
20-29	102 (93.6%)	7 (6.4%)	
30-34	116 (88.5%)	15 (11.5%)	
35-39	107 (95.5%)	5 (4.5%)	
> 40	44 (93.6%)	3 (6.4%)	
Maternal ethnicity			.123
Other	58 (87.8%)	8 (12.2%)	
White	310 (93.4%)	22 (6.6%)	
Maternal BMI at first prenatal visit	34.5 (4.3)	37.6 (6.5)	.001

Abbreviations: AGA, appropriate for gestational age; BMI, body mass index; GDM, gestational diabetes mellitus; GW, gestational weeks; HbA_{1c}, glycated hemoglobin A_{1c}; LGA, large for gestational age; OGTT, oral glucose tolerance test; pGCD59, plasma glycated CD59; SGA, small for gestational age; SPU, standard peptide units.

[a] Chi-square test for proportions and one-way analysis of variance for means.

[b] HbA_{1c}: 11.3% missing.

[c] C-peptide: 48.9% missing.

Infants with NH were more likely to have a lower birth weight (3309.3 vs 3477 g; $P = .014$) and be born preterm ($P = .03$). There was a statistically significant difference in proportions of hypoglycemia across categories of birth weight, with SGA and LGA infants being more likely to develop NH ($P = .001$).

There were no statistically significant statistical differences in mean pGCD59 levels by NH status across birth weight categories, prematurity, maternal HbA_{1c}, maternal age, and BMI (Table 2). Mean pGCD59 values were statistically significantly higher in pGCD59 tertile 3 (4.5 vs 3.9 SPU; $P < .001$) and in the FPG tertile 3 (3.9 vs 3.2 SPU; $P < .001$) in the NH subgroup compared to infants without NH. There were statistically significantly higher levels of pGCD59 in women with GDM (< 20 weeks of gestation) who had infants with NH ($P < .001$) compared to women without GDM with infants with NH. We also found evidence of a strong association between pGCD59 and NH for maternal ethnicity (one-way analysis of variance; $P < .001$).

Table 2.

Mean pGCD59 by neonatal hypoglycemia status less than 20 gestational weeks (n = 399)

	Neonatal hypoglycemia		
	No, n (%)	**Yes, n (%)**	
	n = 369	**n = 30**	
Neonatal variables	**Mean GCD59 (SD)**	**Mean GCD59 (SD)**	***P*[a]**
Birth weight, g			.377
LGA	3.1 (1.2)	2.9 (1.5)	
AGA	2.7 (0.8)	3.3 (1.3)	
SGA	2.6 (0.8)	3.2 (1.3)	
Prematurity (< 37 GW)			.063
Yes	2.4 (0.5)	2.9 (1.0)	
No	2.7 (0.8)	3.2 (1.3)	
Maternal variables			
Tertile maternal pGCD59, SPU			< .001
Tertile 1 (≤ 2.5)	2.0 (0.4)	2.0 (0.3)	
Tertile 2 (> 2.5 ≤ 3.2)	2.9 (0.2)	2.7 (0.2)	
Tertile 3 (> 3.2)	3.9 (0.7)	4.5 (0.7)	
Tertile HbA_{1c} [b], % (mmol/mol)			.224
Tertile 1 (≤ 5.1 (32))	2.7 (0.7)	2.9 (1.0)	
Tertile 2 (> 5.1 (32) ≤ 5.5 (37))	2.7 (0.9)	2.7 (1.3)	
Tertile 3 (>5.5 (37))	2.8 (0.9)	3.2 (1.3)	
Tertile FPG, mmol/L			< .001
Tertile 1 (≤ 4.4)	2.4 (0.5)	1.9 (0.4)	
Tertile 2 (> 4.4 ≤ 4.7)	2.7 (0.8)	3.0 (1.1)	
Tertile 3 (> 4.7)	3.2 (0.9)	3.9 (1.1)	
Maternal GDM status			< .001
No	2.6 (1.0)	2.5 (1.0)	
Yes	3.7 (0.7)	3.8 (1.1)	
Maternal age in categories, y			.447
20-29	2.7 (0.9)	2.9 (1.1)	
30-34	2.8 (0.8)	3.4 (1.4)	
35-39	2.7 (0.7)	3.1 (1.1)	
> 40	2.9 (0.8)	2.9 (1.7)	

Abbreviations: AGA, appropriate for gestational age; BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; GW, gestational weeks; HbA_{1c}, glycated hemoglobin A_{1c}; LGA, large for gestational age; pGCD59, plasma glycated CD59; SGA, small for gestational age; SPU, standard peptide units.

[a] Analysis of variance.

[b] HbA_{1c}: 11.3% missing.

The odds of NH were approximately 2-fold higher in infants from mothers in the third compared to those from mothers in the first tertile of pGCD59 values (ie, OR 2.41; 95% CI, 1.03-5.63). However, in multivariable analysis, adjusted for maternal BMI, age, and ethnicity, higher pGCD59 values were not statistically significantly associated with the prevalence of NH (Table 3). Maternal BMI but not maternal age or ethnicity was associated with NH. For each 5-unit BMI increase there was 60% higher odds of NH. The AUC computed from model 4 was 0.70 (95% CI, 0.56-0.78) (Fig. 1). Similar but slightly lower results were obtained for HbA_{1c} with a generated adjusted AUC of 0.64 (95% CI, 0.51-0.76) and FPG, which generated an AUC of 0.68 (95% CI, 0.57-0.78) (see Table 3 and Fig. 1). The odds of NH were 7 times higher in infants of mothers with GDM diagnosed at less than 20 weeks of gestation compared to those of mothers without GDM (ie, OR 7.3; 95% CI, 3.33-16.17) (see Table 3). The adjusted AUC generated by GDM status (< 20 weeks of gestation) for NH was 0.74 (95% CI, 0.67-0.86) (see Table 3 and Fig. 1).

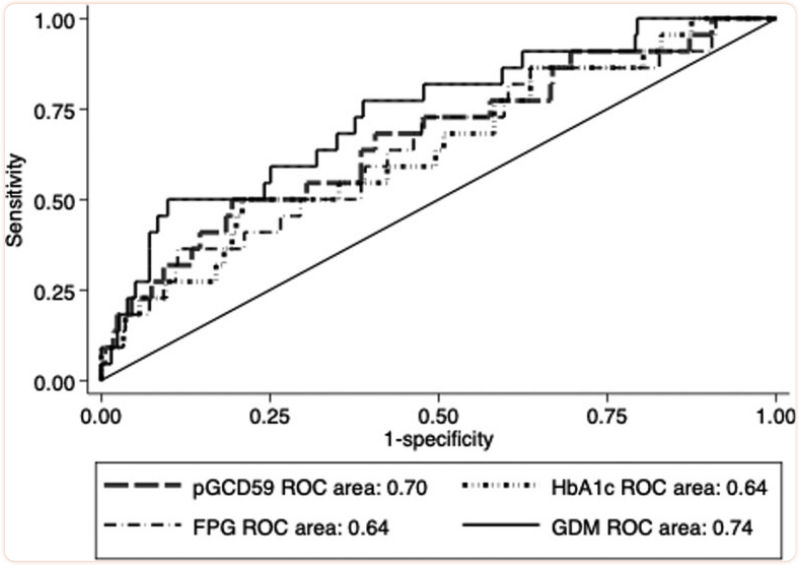

Figure 1.

Comparative predictive performance of maternal plasma glycated CD59 (pGCD59), maternal glycated hemoglobin A_{1c} (HbA_{1c}), maternal fasting plasma glucose (FPG), and gestational diabetes mellitus (GDM) status at less than 20 gestation weeks using receiver operating characteristic curves (ROC) for neonatal hypoglycemia (n = 399).

Table 3.

Neonatal hypoglycemia risk by A, plasma glycated CD59; B, glycated hemoglobin $A1_c$; C, fasting plasma glucose tertile; and D, gestational diabetes mellitus status at less than 20 gestational weeks adjusted for maternal age, ethnicity, and body mass index (n = 399)

A: Variables	**Model 1**	**Model 2**	**Model 3**	**Model 4**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Tertile pGCD59, SPU				
Tertile 2 vs tertile 1	0.62 (0.22-1.71)	0.70 (0.25-1.97)	0.71 (0.25-1.99)	0.65 (0.23-1.86)
Tertile 3 vs tertile 1	2.41 (1.03-5.63)	2.28 (0.96-5.43)	2.30 (0.96-5.45)	2.12 (0.87-5.16)
Maternal BMI				
Per 5-unit increase		1.66 (1.19-2.33)	1.66 (1.18-2.32)	1.63 (1.16-2.29)
Maternal age, y				
Per 5-y increase			0.93 (0.65-1.35)	0.94 (0.65-1.35)
Maternal ethnicity				
Other vs White				1.56 (0.62-3.90)
No.	399	397	397	396
Model AUC (95% CI)	0.64 (0.54-0.74)	0.69 (0.58-0.79)	0.69 (0.58-0.79)	0.70 (0.56-0.78)
B: Variables	Model 1	Model 2	Model 3	Model 4
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Tertile HbA_{1c}, %				
Tertile 2 vs tertile 1	1.26 (0.37-4.26)	1.22 (0.35-4.13)	1.22 (0.36-4.16)	1.19 (0.35-4.08)
Tertile 3 vs tertile 1	2.48 (0.89-6.93)	1.96 (0.68-5.67)	1.97 (0.68-5.67)	1.88 (0.64-5.52)
Maternal BMI				
Per 5-unit increase		1.54 (1.03-2.30)	1.54 (1.02-2.30)	1.49 (0.99-2.26)
Maternal age, y				
Per 5-y increase			1.02 (0.67-1.55)	1.02 (0.67-1.54)
Maternal ethnicity				
Other vs White				1.70 (0.61-4.71)
No.	360	359	359	358
Model AUC (95% CI)	0.60 (0.48-0.72)	0.64 (0.53-0.76)	0.64 (0.53-0.76)	0.64 (0.51-0.76)
C: Variables	Model 1	Model 2	Model 3	Model 4
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Tertile FPG, mmol/L				
Tertile 2 vs tertile 1	1.51 (0.53-4.30)	1.43 (0.49-4.10)	1.46 (0.50-4.24)	1.45 (0.50-4.19)
Tertile 3 vs tertile 1	2.59 (1.02-6.57)	2.23 (0.86-5.76)	2.28 (0.88-5.91)	2.18 (0.83-5.70)
Maternal BMI				

Abbreviations: AUC, area under the curve; BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; HbA_{1c}, glycated hemoglobin A_{1c}; OR, odds ratio; pGCD59, plasma glycated CD59; Ref., reference; SPU, standard peptide units.

We also assessed the association between pGCD59, and umbilical cord C-peptide levels (Fig. 2), and we found a positive linear association between the 2 variables (R = 0.23).

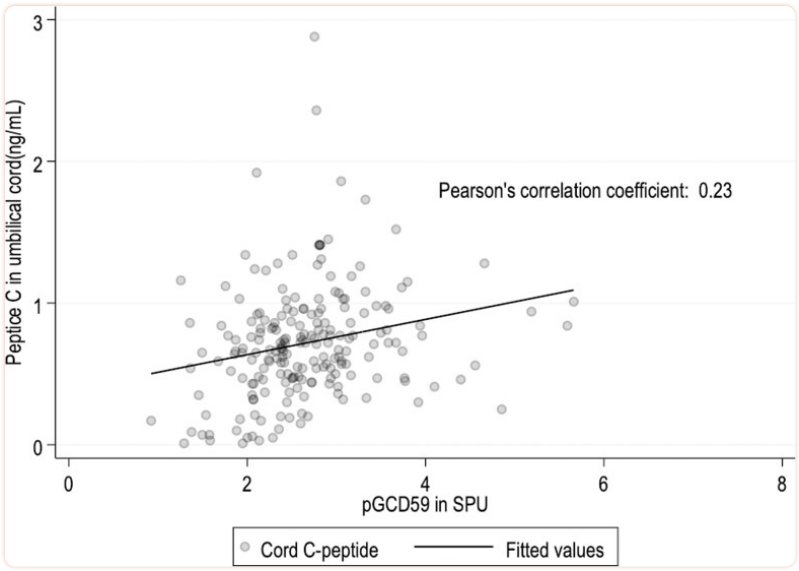

Figure 2.

Association between cord C-peptide (ng/mL) and maternal plasma glycated CD59 (pGCD59) in standard peptide units (SPU) (n = 204, cord C-peptide 8.9% missing).

Discussion

This study has shown that higher maternal levels of pGCD59 in early pregnancy (< 20 weeks' gestation) are associated with NH, but this association was attenuated when adjusted for maternal BMI.

Recently, Meek et al (23) studied the association between pGCD59 and pregnancy complications in pregnant women with type 1 diabetes (24). The team found that pGCD59 levels in each trimester of pregnancy were associated with NH. pGCD59 in the first trimester could predict NH with an unadjusted AUC of 0.61 and in the second trimester with an AUC of 0.72. This is consistent with our findings that pGCD59 predicts the development of NH with an unadjusted AUC of 0.64. The mean pGCD59 levels in the Meek study in the first trimester to second trimester ranged from 7.07 (SD 4.84) to 7.15 (SD 4.76) SPU, while in our study they ranged from 2.7 (SD 0.8) to 3.2 (SD 1.3) SPU, reflecting the higher degree of hyperglycemia in women with type 1 diabetes, as expected.

We found higher rates of NH in specific high-risk subgroups (high maternal BMI, SGA, LGA) in our cohort, and these findings are supported by other studies.

The link between maternal BMI and NH was documented more than 30 years ago (25). Callaway et al (26), in a study that included 14 230 women, found an increased risk of NH of 2.57 (95% CI, 1.94-3.32) in the obese category and of 7.14 (95% CI, 3.04-16.74) in the morbidly obese category (BMI > 40). García-Patterson et al (27) found that BMI is an independent predictor for NH, women with a prepregnancy BMI greater than 25 having double the risk of delivering an infant that will develop NH. This supports our findings of higher NH rates in the higher BMI category and the increased NH risk with the increase of BMI.

Zhao et al (28) found that birth weight, poor feeding, and mother's GDM status are statistically significantly associated with NH. Doctor et al (29) found that SGA infants are 5 times more likely to develop hypoglycemia compared to AGA infants. The link between low birth weight and infant hypoglycemia has been confirmed in other studies as well (30-32). This is most likely due to decreased energy deposits and perinatal compromise. Our findings reveal higher NH rates in SGA infants compared to AGA, supporting previous findings.

pGCD59 has been shown to display high sensitivity and specificity in the diagnosis of GDM at 24 to 28 weeks' gestation and to be positively associated with higher prevalence of LGA infants (4). More so, on a recent analysis of samples from the DALI study, pGCD59 levels (< 20 weeks' gestation) were found to be associated with early pregnancy GDM (< 20 weeks' gestation) with an AUC of 0.86 and were strongly associated with high prevalence and odds for delivering an LGA baby (5). The scientific community has explored the link between LGA and NH. Voormolen et al (33) found that LGA infants had a 2-fold risk for developing severe NH (OR 1.93; 95% CI, 1.19-3.14), supporting previous published results (34). Alemu (35) found an almost 3-fold increased risk of hypoglycemia in LGA babies (OR 2.9; 95% CI, 2.81-2.94) in a general population. Our study found 4 times higher rates of NH in LGA infants compared to AGA. Therefore, a biomarker associated with the prevalence of LGA could also be associated with NH. Given that pGCD59 is a glycation biomarker and is associated with hyperglycemia, it is reasonable to hypothesize that pGCD59's association with NH is a reflection of early pregnancy maternal hyperglycemia.

There are numerous studies indicating an association between hyperglycemia and/or GDM status and infant hypoglycemia (28, 36). Our study observed that higher mean levels of pGCD59, HbA_{1c}, fasting glucose, 1-hour and 2-hour glucose levels, and GDM status (OGTT < 20 weeks of gestation) were statistically significantly associated with higher rates of NH. More so, pGCD59 tertile 3 was associated with higher rates of NH compared to the other tertiles. Our study found a statistically significant difference in mean pGCD59 by NH across GDM status with no difference in mean pGCD59 by NH for maternal age and BMI or birth weight categories. These findings highlight the association between pGCD59 levels and GDM status/hyperglycemia and that pGCD59 association with NH rates might be a reflection of glycemic levels and their effect on developing NH. This is further emphasized by the generated AUCs. pGCD59 generated a better AUC than HbA_{1c} and FPG but slightly worse than GDM status. This suggests that in early pregnancy pGCD59 performs better than HbA_{1c} and FPG in predicting NH, but GDM status is a stronger predictor. The reason for this might be that, in early pregnancy, the levels of glycemia are too low or there has not been sufficient time to generate pGCD59. Assessment at 24 to 28 weeks of gestation might provide better information on pGCD59 ability to predict NH, especially if the mechanism of NH projection is hyperglycemia. As shown in the study by Meek and colleagues (23), pGCD59 showed better predictive power for NH in the second trimester compared to first trimester and this might be related to the degree of glycemia women display mid-pregnancy.

In women with diabetes, cord C-peptide has been associated with NH (37, 38) as a reflection of poor glycemic control and fetal hyperinsulinemia. In our study, in a high-risk population, we did not find higher C-peptide levels in the NH subgroup compared to infants without NH; however, we did find a positive linear association between pGCD59 levels and cord C-peptide levels suggesting that the association between pGCD59 and NH could be mediated through maternal glycemia.

Our study also found a statistically significant difference between mean pGCD59 by NH for maternal ethnicity, with mothers of other ethnic background exhibiting higher mean pGCD59 levels compared to the White ethnicity subcategory. Differences between glycemic markers by race have previously been observed, differences that cannot entirely be explained by differences in sociodemographic, anthropomorphic variables, treatment adherence, and quality of care (39). Genetic polymorphism and nonglycemic mechanisms have been proposed as possible explanations but require further exploration (40).

This was a secondary study using frozen Biobanked samples from the DALI study. One of the eligibility criteria for the DALI study was a BMI greater than or equal to 29, and including only overweight and obese women limits the generalizability of the results. The study had a small number of NH cases; a larger cohort of NH cases would allow for more complex and accurate subcohort analysis. No data were collected on the type of device used for glucose assessment, each center collecting glucose measurements in accordance with local protocols. All glucose samples were collected less than 48 hours post delivery; however no additional data were collected on the exact timing of glucose sampling. Furthermore, this study included a high number of White participants with a low number of other ethnicities, making the generalizability of results to other ethnicities difficult.

Conclusion

The results of this study indicate that pGCD59 levels measured early in pregnancy (< 20 weeks' gestation) in a high-risk population can identify infants at risk of developing NH with only fair discrimination on adjusted models. Further prospective studies are required in a larger population, across all BMI categories, with additional assessment of pGCD59 levels later in pregnancy, at the time of routine GDM screening (24-28 weeks' gestation). Further studies are also needed to assess the biomarker's predictive power for NH in a larger GDM-only population.

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Abbreviations

AGA	appropriate for gestational age
AUC	area under the curve
BMI	body mass index
DALI	Vitamin D and Lifestyle Intervention for Gestational Diabetes
ELISA	enzyme-linked immunosorbent assay
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
GW	gestational weeks
HbA_{1c}	glycated hemoglobin A_{1c}
LGA	large for gestational age
NH	neonatal hypoglycemia
OGTT	oral glucose tolerance test
pGCD59	plasma glycated CD59
ROC	receiver operating characteristic
SGA	small for gestational age
SPU	standard peptide units

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Author Contributions

D.B., M.T.C., M.A.L.F., and J.A.H. analyzed the data; D.B., G.D., and F.D. provided the study samples on behalf of the DALI Study Group and contributed to data analysis and to writing the manuscript. All authors (D.B., M.A.L.F., M.T.C., G.D., P.O.S., F.D., J.A.H., and the DALI Core Investigator Group) made substantial contributions to the interpretation of data, revised the manuscript critically for important intellectual content, and approved the final version to be published. D.B. is responsible for the integrity of the work as a whole.

Disclosures

J.A.H. has a financial interest in Mellitus, LLC. Mellitus is developing diagnostic tools for diabetes. J.A.H.'s interests were reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict-of-interest policies. M.T.C., M.A.L.F., D.B.,

G.D., P.O.S., and F.D. have nothing to disclose.

Data Availability

The data supporting the findings are available on reasonable request.

Clinical Trial Information

DALI trial registration number ISRCTN70595832 (registered November 21, 2011).

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