



Plasma Glycated CD59, a Novel Biomarker for Detection of Pregnancy-Induced Glucose Intolerance

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Pamela Ghosh,¹
 Miguel A. Luque-Fernandez,^{2,3}
 Anand Vaidya,⁴ Dongdong Ma,¹
 Rupam Sahoo,¹ Michael Chorev,¹
 Chloe Zera,⁵ Thomas F. McElrath,⁵
 Michelle A. Williams,³ Ellen W. Seely,⁴ and
 Jose A. Halperin¹

OBJECTIVE

Plasma glycated CD59 (pGCD59) is an emerging biomarker in diabetes. We assessed whether pGCD59 could predict the following: the results of the glucose challenge test (GCT) for screening of gestational diabetes mellitus (GDM) (primary analysis); and the diagnosis of GDM and prevalence of large for gestational age (LGA) newborns (secondary analyses).

RESEARCH DESIGN AND METHODS

Case-control study of 1,000 plasma samples from women receiving standard prenatal care, 500 women having a normal GCT (control subjects) and 500 women with a failed GCT and a subsequent oral glucose tolerance test (case patients).

RESULTS

Compared with control subjects, the median (interquartile range) pGCD59 value was 8.5-fold higher in case patients and 10-fold higher in GDM patients, as follows: control subjects 0.33 (0.19); case patients 2.79 (1.4); GDM patients 3.23 (1.43) ($P < 0.001$); area under the receiver operating characteristic curve 0.92. LGA prevalence was 4.3% in the lowest quartile and 13.5% in the highest quartile of pGCD59.

CONCLUSIONS

One pGCD59 measurement during weeks 24–28 identifies pregnancy-induced glucose intolerance with high sensitivity and specificity and can potentially identify the risk for LGA.

Screening for gestational diabetes mellitus (GDM) with an oral glucose challenge test (GCT) is a standard of care for all nondiabetic pregnant women (1,2) because the adverse pregnancy outcomes associated with GDM can be mitigated with appropriate therapy (3,4). Screening (GCT) and diagnostic oral glucose tolerance tests (OGTTs) are time consuming and uncomfortable, and have poor reproducibility (5). Other tests such as those for measurement of HbA_{1c} or fructosamine are not routinely conducted during prenatal care because of their low sensitivity and specificity in identifying women who are at risk for GDM (6,7).

The complement system and its regulators reportedly play a role in the pathogenesis of diabetes complications (8). In diabetes, nonenzymatic glycation inactivates the complement inhibitor CD59, forming glycated CD59 (GCD59) (9). Using a sensitive and specific ELISA for GCD59 in blood, we have shown that plasma GCD59 (pGCD59) levels are significantly higher in individuals with type 2 diabetes and independently predict the response to the OGTT (10).

¹Division of Hematology, Brigham and Women's Hospital, Boston, MA

²Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, U.K.

³Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA

⁴Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital, Boston, MA

⁵Division of Maternal Fetal Medicine, Brigham and Women's Hospital, Boston, MA

Corresponding author: Jose A. Halperin, jhalperin@bwh.harvard.edu.

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P.G. and M.A.L.-F. contributed equally to this work.

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Our primary objective was to assess the accuracy of pGCD59 in predicting the results of the GCT. As secondary aims, we assessed the accuracy of pGCD59 in predicting the diagnosis of GDM by OGTT and explored the association of pGCD59 with the prevalence of large for gestational age (LGA) newborns at delivery.

RESEARCH DESIGN AND METHODS

We performed a case-control study measuring pGCD59 in 1,000 samples from women undergoing routine two-step GDM screening and diagnosis at Brigham and Women's Hospital (BWH; 2012–2014). Two sets of 500 samples each were collected randomly from women who either passed the 50-g GCT and therefore did not undergo a 3-h OGTT (control subjects) or failed the GCT and therefore underwent a standard-of-care 100-g, 3-h OGTT (case patients) at BWH. The gestational week at the time of sample collection was the same for control subjects and case patients (26.5 ± 3.3 and 26 ± 1.8 , respectively). Samples for pGCD59 measurement were separated from the same tubes used to measure plasma glucose and were stored (-80°C) by Partners HealthCare Crimson Biorepository Core (10), a clinical investigation facility that anonymously collects discarded materials from the clinical laboratories of Partners HealthCare Hospitals. Medical information was retrieved from electronic records before samples were deidentified; only coded samples were delivered for pGCD59 measurement. pGCD59 was measured using the specific ELISA described in the study by Ghosh et al. (11); test operators were blind to the women's glucose status. The interassay coefficient of variation was $<10.0\%$. The Partners HealthCare Institutional Review Board approved this study (Protocol 2011P002254/BWH). We followed the Standards for Reporting of Diagnostic Accuracy guidelines for study design and reporting.

Statistical Analysis

Patient characteristics were described using medians and interquartile ranges for continuous variables and count proportions for categorical variables. The sensitivity and specificity of pGCD59 to predict the results of the GCT were assessed using nonparametric estimates of the receiver operating characteristic (ROC) curves and

respective area under the ROC curve (AUROC) (12). Positive and negative predictive values (PPV and NPV) and positive likelihood ratio (LR+) were calculated as in (13). Following World Health Organization recommendations, LGA was defined as ≥ 90 th percentile birth weight adjusted for gestational age at delivery and determined from the latest sex-specific reference curves derived from a large sample that reflects the ethnic distribution of the U.S. population (14). Statistical analyses were performed using Stata

software version 13.1 (StataCorp, College Station, TX).

RESULTS

Among the 500 case patients, 127 met Carpenter and Coustan (C&C) criteria for GDM (15). Supplementary Table 1 summarizes maternal and infant characteristics; the ethnic/racial composition of the study subjects was comparable to that of the U.S. population (14). Median pGCD59 levels were as follows: 8.5-fold higher in the 500 case patients than in the 500 control

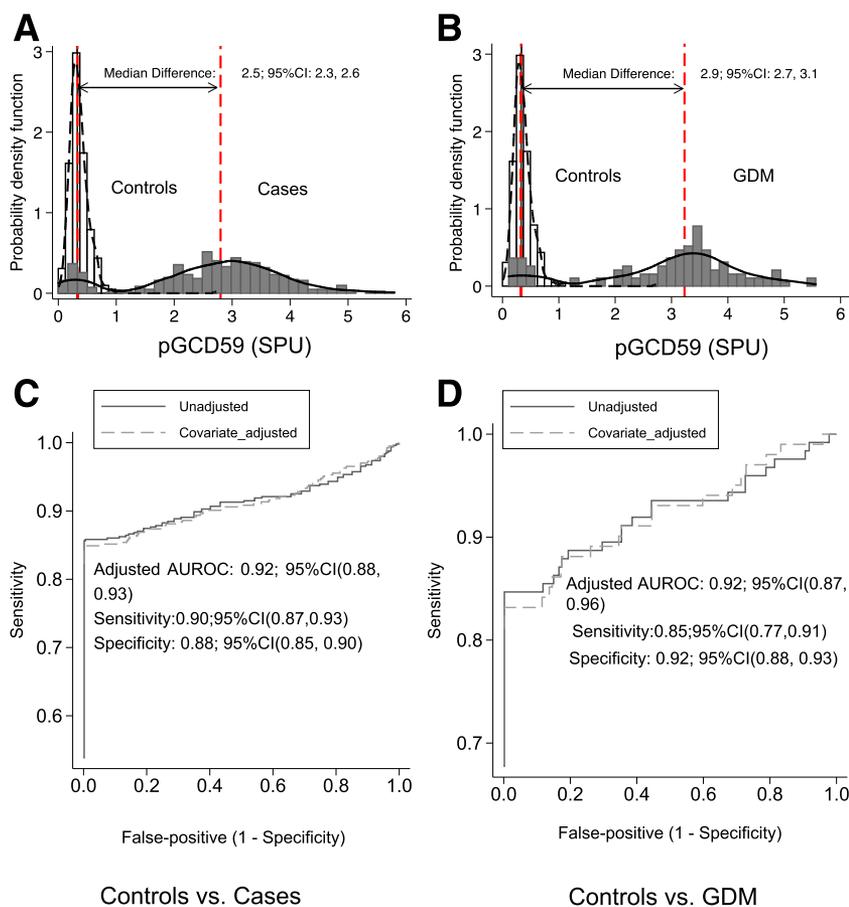


Figure 1—A and B: pGCD59 probability density functions by case-control status and between control subjects vs. GDM. Glucose challenge tests were adjudicated using American Congress of Obstetricians and Gynecologists guidelines: failed 50-g GCT, ≥ 140 mg/dL; 100-g, 3-h OGTT: no GDM, zero or one abnormal glucose value; GDM, two or more abnormal glucose values based on C&C criteria. A: Control subjects vs. case patients. B: Control subjects vs. GDM patients. The red dotted lines indicate the median pGCD59 values for the respective groups; the difference in median values between two groups and 95% CIs are mentioned in the figure ($n = 1,000$). C and D: ROC curve AUCs by case-control status and control subjects vs. GDM patients. C: Control subjects vs. case patients. D: Control subjects vs. GDM patients. Marginal and conditional ROC curves were computed and adjusted for maternal age, BMI, race/ethnicity, multiplicity, and gestational age at GCD59 determination and history of diabetes. AUROCs were derived using the DeLong et al. (20) nonparametric tied corrected estimator, and the percentile values of the case patient observations with respect to the control distribution were used to derive the tied corrected estimator (20). Under nonparametric estimation, SEs and derived AUROCs and 95% CIs were estimated using cross-validation and bootstrapping procedures with 1,000 replications. Dashed lines, ROC curves adjusted for maternal age, race/ethnicity, BMI, gestation week at pGCD59 determination, and a history of diabetes ($n = 1,000$); insets, adjusted AUC, sensitivity, and specificity with 95% CI; solid lines, unadjusted ROC curves.

subjects and 10-fold higher in the 127 case patients in whom GDM was diagnosed by 3-h OGTT (Fig. 1, Supplementary Fig. 1, and Supplementary Table 2). The probability density function (Fig. 1A and B) and AUROCs (Fig. 1C and D) show that pGCD59 independently discriminated case patients from control subjects with high sensitivity and specificity, even after adjustment for covariates such as maternal age, BMI, race/ethnicity, multiplicity, gestational age, and history of diabetes (adjusted AUROC control subjects vs. case patients 0.92 [95% CI 0.88, 0.93]; adjusted AUROC control subjects vs. GDM 0.92 [95% CI 0.87, 0.96]). Positive and negative predictive values for the overall distribution of GCD59 values to identify case patients were 99.3% (95% CI 97.9, 99.8) and 87.5% (95% CI 84.5, 90.1); and 99.1% (95% CI 94.9, 99.9) and 95.6% (95% CI 93.8, 97.4) to identify women with GDM. Women with pGCD59 values \geq 6th decile had a likelihood of having a failed GCT that was eightfold higher than for those women with pGCD59 values <6th decile (positive likelihood ratio 7.97) (Supplementary Table 3).

Among the 852 singletons who had recorded birth weight and sex, 86 (10%) were identified as LGA, 28 of which were born to control subjects and 58 of which were born to case patients (Supplementary Table 1). Higher maternal pGCD59 level was associated with a higher prevalence of LGA, which was 4.3% (9 of 207 women) in the lowest quartile of pGCD59 and was 13.5% (29 of 214 women) in the highest quartile of pGCD59 (χ^2 test, $P < 0.0001$) (Supplementary Fig. 2). This result was not affected by adjustment for maternal age, race/ethnicity, and BMI. Notably, 45 of 58 LGA infants (78%) in the case population were born to mothers who did not meet C&C OGTT threshold criteria for GDM but had median pGCD59 values that were sevenfold higher than those for control subjects (Supplementary Table 4).

CONCLUSIONS

This study explored the clinical utility of pGCD59 to screen/diagnose GDM. One maternal pGCD59 measurement at a mean gestational week \sim 26 predicted the results of the GCT with high sensitivity and specificity and independently of covariates such as age, BMI, race/ethnicity, multiplicity, gestational age, and history of diabetes (Fig. 1 and Supplementary

Fig. 1). Median pGCD59 values were progressively higher across the categories of maternal glucose tolerance (Supplementary Table 2). These findings indicate that pGCD59 potentially represents a convenient and effective alternative to the cumbersome glucose challenge methods currently used to screen/diagnose GDM.

Glucose tolerance tests fail to recognize the continuous association between maternal hyperglycemia and abnormal pregnancy outcomes and exclude milder forms of glucose intolerance that may still impart perinatal risk (16,17). The progressively higher pGCD59 levels observed across the GCT-OGTT categories (Supplementary Table 2) suggest that pGCD59 may reflect the continuum of pregnancy-induced glucose intolerance described by the seminal Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study (16).

pGCD59 levels at gestational week \sim 26 were associated with a higher prevalence of LGA at birth. Among case patients, 22% of LGA newborns were born to women in whom GDM was diagnosed, whereas 78% of LGA newborns were born to women who failed the GCT but did not meet the C&C criteria for GDM. This likely reflects the effect of treatment on women who have received a formal diagnosis of GDM and is consistent with conclusions of the HAPO and other studies showing that women in an intermediate category between “normal” and “abnormal” glucose tolerance are at higher risk of abnormal pregnancy outcomes (18). Currently, there are no guidelines for the management of women in the intermediate category, and, therefore, their management is the same as that for women with a normal GCT result. The fact that the 45 case patients who did not meet C&C criteria for GDM but delivered LGA newborns had median pGCD59 levels that were sevenfold higher than those for control subjects provides additional evidence for the potential clinical utility of pGCD59 for screening for/diagnosis of GDM (16).

The limitations of the study are as follows: 1) the study was observational; 2) clinical and demographic characteristics were limited to those available in medical records; 3) we could not adjust for the time of day when the GCT was performed since all testing was performed per routine clinical care (19); and 4) the study was not aimed at establishing a clinically useful cutoff value or assessing

how pGCD59 measures might influence clinical care in real time or the impact of treatment on the prevalence of LGA.

In summary, this is the first study showing that a single measurement of pGCD59 at gestational week \sim 26 represents a simplified method for identifying women who would have failed a GCT and are at higher risk of GDM and possibly of having an LGA newborn. The validation of pGCD59 as a biomarker for the detection of pregnancy-induced glucose intolerance and determination of clinically useful cutoff values will require multicenter studies and “consensus” expert committees that will take into account relative risks, cost/benefits, and other individual and public health considerations, as has been the norm with currently used methodologies for the screening and diagnosis of GDM. Future studies should also assess the following: 1) whether pGCD59 similarly classifies pregnant women with normal or abnormal glucose tolerance as defined by the 2-h, 75-g OGTT recommended by the International Association of the Diabetes and Pregnancy Study Groups; 2) whether pGCD59 is a predictor of adverse outcomes in pregnant women in the intermediate category of glucose tolerance who might benefit from treatment; and 3) whether pGCD59 detects glucose intolerance earlier in pregnancy than current practice, prompting earlier interventions that may mitigate further the risks associated with maternal hyperglycemia.

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Duality of Interest. M.C. and J.A.H. have a financial interest in Mellitus, LLC, which is developing diagnostic tools for diabetes. The interests of M.C. and J.A.H. were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict-of-interest policies. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. P.G. and A.V. participated in the study concept and design; in the acquisition, analysis, or interpretation of the data; and in drafting and critically revising the manuscript for important intellectual content. M.A.L.-F. participated in the acquisition, analysis, or interpretation of the data and in drafting and critically revising the manuscript for important intellectual content and performed the statistical analysis. D.M. and R.S. participated in critically revising the manuscript for important intellectual content. M.C. participated in drafting and critically revising the manuscript for important intellectual content.

C.Z., T.F.M., and E.W.S. participated in the study concept and design and in critically revising the manuscript for important intellectual content. M.A.W. performed the statistical analysis and participated in critically revising the manuscript for important intellectual content. J.A.H. participated in the study concept and design; in the acquisition, analysis, or interpretation of the data; and in drafting and critically revising the manuscript for important intellectual content and supervised the study. P.G. and J.A.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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