Original Article
Clinical Chemistry

Ann Lab Med 2019;39:524-529
https://doi.org/10.3343/alm.2019.39.6.524
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Screening for Gestational Diabetes Mellitus by Measuring Glycated Hemoglobin Can Reduce the Use of the Glucose Challenge Test

Jose-Maria Maesa, Ph.D., Patricia Fernandez-Riejos, Ph.D., Concepcion Gonzalez-Rodriguez, M.D., and Victor Sanchez-Margalet, M.D.

Department of Clinical Biochemistry, Macarena University-Hospital, Seville, Spain

Background: Physiological changes during pregnancy, such as dilutional anemia and a reduced half-life of red blood cells, have prevented the use of glycated Hb (HbA_{1c}) as a biomarker for gestational diabetes mellitus (GDM). Nevertheless, increasing evidence supports the use of HbA_{1c} in GDM diagnostic strategies.We studied HbA_{1c} as a biomarker of GDM and its possible use as a screening test to avoid the use of the glucose challenge test (GCT).

Methods: This case-control study involved 607 pregnant women between the 24th and 28th week of gestation. HbA_{1c} level was determined, and GDM was diagnosed according to the National Diabetes Data Group criteria. The area under the ROC curve (AUC) was determined; two low and two high cut-off points were established to rule out GDM and classify high-risk pregnant women, respectively. For each cut-off, sensitivity (S), specificity (SP), and total number and percentage of GCTs avoided were determined.

Results: The AUC for HbA_{1c} diagnostic performance was 0.68 (95% confidence interval 0.57–0.79). Using 4.6% HbA_{1c} (27 mmol/mol) as the lower cut-off (S=100%), 14% of participants could avoid the GCT. Using 5.5% HbA_{1c} (36 mmol/mol) as the upper cut-off (SP=94.5%), 6% of participants would be considered at high risk.

Conclusions: HbA_{1c} can be used as a screening test prior to the GCT, thereby reducing the need for the GCT among pregnant women at a low risk of GDM.

Key Words: Gestational diabetes mellitus, Glycated Hb, Glucose challenge test, Screening

Received: November 17, 2018
Revision received: April 5, 2019
Accepted: June 11, 2019

Corresponding author:
Jose-Maria Maesa, Ph. D.
Department of Clinical Biochemistry, Macarena University-Hospital, Pascual de Gayangos 41, E, 2ºI, 41002, Seville, Spain
Tel: +34-666121496
Fax: +34-954907048
E-mail: jmaesam@gmail.com

INTRODUCTION

The diagnosis of gestational diabetes mellitus (GDM) is not yet completely and universally resolved. Despite the latest attempt to universalize diagnostic criteria by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [1], with the support of the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study [2], a consensus has not yet been reached. A systematic review carried out by the Cochrane Library concluded that there is not enough evidence to recommend any strategy universally, and that each one should be applied according to the risks of the population to be treated [3].

In many countries, a two-step strategy is used following the American Diabetes Association (ADA) [4] and American College of Obstetrician and Gynecologists (ACOG) criteria [5]. The strategy begins with universal screening based on the glucose challenge test (GCT), followed by an oral glucose tolerance test (OGTT) of 100 g and four blood extractions for pregnant women with a GCT value ≥140 mg/dL.

The performance of the OGTT as a diagnostic test is very high;

however, the sensitivity (S) of the GCT ranges between 70% and 88% for the 140 mg/dL cut-off point [6]. In addition, the reproducibility of the OGTT is low [7], especially when compared with that of other biomarkers such as glycated Hb (HbA_{1c}) [8]. Furthermore, it is an important source of expense for laboratories and causes great inconvenience to pregnant women, as they must spend at least one hour in the hospital for the GCT. In addition, the test itself causes discomfort and is frequently associated with nausea and vomiting [3]. Researchers have looked for other parameters that can be tested so as to replace the GCT or at least reduce the number of pregnant women subjected to the GCT. One possible biomarker, HbA_{1c}, is currently used as a fundamental parameter for the diagnosis and monitoring of diabetes mellitus [10, 11].

However, the dilutional anemia associated with pregnancy and the reduction in the half-life of red blood cells (also characteristic of pregnancy) have prevented the extension of HbA_{1c} reference ranges from non-pregnant to pregnant women. Nevertheless, many HbA_{1c} characteristics could make it a very useful biomarker of GDM. For example, it has a clear correlation with average glycemia during the three to four months prior to measurement; it is standardized and has great precision andreliability; its determination is routine in most clinical laboratories; and the procedure is very simple for the patient, without the need to fast.

Research supports the use of HbA_{1c} as a biomarker of GDM. The United States Preventive Service Task Forces commissioned a systematic review [6] that included four studies proposing the use of HbA_{1c} as a screening test for GDM. The HAPO study also established a statistically significant relationship between HbA_{1c} and a series of pregnancy complications [11]. However, the evidence is still insufficient, and clinical guidelines have not incorporated HbA_{1c} in relation to GDM, except as a marker of pregestational diabetes [4]. We provide new results that support the use of HbA_{1c} as a screening and diagnostic tool for GDM. To the best of our knowledge, this is the first study to examine the use of HbA_{1c} as a screening test in the context of the National Diabetes Data Group (NDDG) strategy [12].

METHODS

Study design and population

This retrospectivecase-control study was conducted between December 1, 2016 and May 20, 2017. We randomly selected 607 pregnant women who attended Macarena University Hospital, Sevilla, Spain, for the GCT. We excluded those who were not between the 24th and the 28th week of gestation and those with a history of diabetes and other pathologies that could interfere with HbA_{1c} determination, such as hemoglobinopathies. Our study was approved by the Research Ethics Committee of the Virgen del Rocío and Virgen Macarena University Hospitals, Seville, Spain (code: PFR-DG-2017-01).

HbA_{1c} measurement

Whole blood samples (5 mL EDTA tubes) were collected from pregnant women at the same time as that for GCT blood extraction. HbA_{1c} was determined in fresh samples, using G8 (Tosoh Corporation, Shiba-Mianto-ku, Tokyo, Japan), an HPLC ion exchange, preceded by a pre-filter to avoid the rapid deterioration of the column, with a detection system comprising a photometer that quantifies the different Hb fractions identified by their specific retention times.

The instrument was calibrated according to the manufacturer's specifications, and we performed daily controls with the two internal controls provided by the manufacturer and three levels of the Liquichek Diabetes Control, provided by Bio-Rad (Irvine, CA) and prepared from whole human blood, as an external quality control. Instrument precision is CV=0.65% [13]. It is certified by the National Glycohemoglobin Standardization Program. The GCT values were obtained for all participants, and the OGTT value was measured in participants with a GCT value ≥140 mg/dL, using the Advia 2400 glucose hexokinasemethod (Siemens Healthineers, Erlangen, Germany), calibrated according to the manufacturer's specifications. We performed a daily controlon glucose hexokinase method with two levels of the internal control provided by the manufacturer and an external control provided by the Spanish Society of Clinical Medicine.

GDM diagnosis

We diagnosed GDM following the recommendations of the Spanish Group of Diabetes and Pregnancy [14]; GDM screening is performed for all pregnant women. The screen consists of 50 g of GCT followed by 100 g of OGTT when GCT glycemia is ≥140 mg/dL. The 100 g OGTT follows the recommendations and cut-off points detailed by the NDDG for diagnosing GDM when two or more measures are equal to or greater than the following cut-offs: fasting, 105 mg/dL; 1 hour, 190 mg/dL; 2 hours, 165 mg/dL; and 3 hours, 145 mg/dL [12].

Statistical analysis and diagnostic performance

The Kolmogorov-Smirnov was used to determine the normal distribution of the data. Participants were divided into two groups:

normal and GDM. The mean and SD were calculated for HbA_{1c}, GCT, body mass index (BMI), and age. We determined mean-differences with 95% confidence interval (CI) for these parameters between the two groups. The Student's t-test was used to compare mean HbA_{1c} between groups.

The ROC curve was constructed, and the area under the ROC curve (AUC) with 95% CI was determined. S, specificity (SP), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR), and negative likelihood ratio (-LR) were calculated with 95% CI for four cut-off points, two of which maximize S and two that maximize SP, using IBM SPSS20 (IBM Corp., Armonk, NY, USA).

Assuming a diagnostic strategy in which HbA_{1c} was used as a screening test to avoid the GCT in pregnant women at low risk of GDM (below the lower cut-off), or to perform the confirmatory OGTT in those at high risk (above the higher cut-off) without a previous GCT, we calculated the percentage of participants who would avoid the GCT. In addition, we calculated the false negatives that would occur using the low cut-off points and the negative GCTs that would correspond to the high cut-off points.

RESULTS

HbA_{1c} level, GCT value, age, and BMI were normally distributed. Of 607 participants, 580 formed the normal group (mean age = 31.9±5.8 years, mean BMI = 27.2±4.9 kg/m^2, and mean GCT value = 115.3±27.8 mg/dL). The GDM group included 27 participants (5.4%; mean age = 33.2±4.4 years, mean BMI = 29.5±6.9 kg/m^2, and mean GCT value = 185.6±33.7 mg/dL). As shown in Table 1, HbA_{1c} level differed significantly between groups but age and BMI did not.

Table 2 shows the cut-off points for HbA_{1c} based on the ROC curve. Choosing 4.6% HbA_{1c} (27 mmol/mol) as a low cut-off prevented false negatives in the study population and decreased the number of GCTs performed (86 pregnant women were identified as low-risk) by 14.1%. Using 4.7% HbA_{1c} (28 mmol/mol) as the low threshold resulted in two false negatives and a reduction of 20.8% in the number of GCTs (127 pregnant women

Table 1. HbA_{1c} values in the study population

Parameter*	Normal (N = 580)	GDM (N = 27)	Difference	95% CI	*P*
GCT value (mg/dL)	115.35 (27.81)	185.62 (33.67)	70.26	59.20–81.30	0.00
% HbA_{1c} (mmol/mol)	4.9 (0.30)	5.2 (0.4)	0.20	0.10–0.40	
	31 (3.50)	33 (4.2)	2.70	1.30–4.00	0.00
Age (yr)	31.89 (5.83)	33.19 (4.38)	1.30	-0.93–3.50	0.25
BMI (kg/m^2)	27.20 (4.90)	29.50 (6.90)	2.25	-6.10–6.60	0.18

*All values are summarized as mean (SD).
Abbreviations: HbA_{1c}, glycated Hb; GCT, glucose challenge test; GDM, gestational diabetesmellitus; CI, confidence interval; BMI, body mass index.

Table 2. Cut-off points for HbA_{1c} as a GDM screening test (N = 607, AUC = 0.68 [95% confidence interval: 0.57–0.79])

Cut-off (%HbA_{1c}, mmol/mol)	Rule-out		Rule-in	
	≤4.60, ≤27	≤4.70, ≤28	≥5.50, ≥36	≥5.70, ≥39
S (%) (95% CI)	100 (87.50–100)	92.60 (76.60–97.90)	25.90 (13.20–44.70)	11.10 (3.90–28.10)
SP (%) (95% CI)	14.80 (12.20–18)	21.60 (18.40–25.10)	94.50 (92.30–96.10)	98.80 (97.50–99.40)
PPV (%) (95% CI)	5.18 (3.60–7.40)	5.21 (7.60–3.60)	17.95 (9–32.70)	30 (10.80–60.30)
NPV (%) (95% CI)	100 (95.70–100)	98.43 (94.4–99.60)	96.48 (94.60–97.70)	95.98 (94.1–97.30)
+LR (95% CI)	1.17 (1.14–1.22)	1.18 (1.05–1.32)	4.71 (2.29–9.66)	9.25 (2.52–33.66)
-LR (95% CI)	0	0.34 (0.09–1.32)	0.78 (0.63–0.98)	0.90 (0.79–1.03)
NO GCT, N (%)	86 (14.12)	127 (20.85)	39 (6.40)	10 (1.64)
	False Negative		Negative GCT	
N	0	2	24	6

Abbreviations: HbA_{1c}, glycated Hb; GDM, gestational diabetes mellitus; AUC, area under the curve; S, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio; GCT, glucose challenge test; OGTT, oral glucose tolerance test.

https://doi.org/10.3343/alm.2019.39.6.524

who do not require a GCT). In terms of the high thresholds, 6.4% of pregnant women would directly proceed to a confirmatory OGTT.

DISCUSSION

We proposed inclusion of HbA_{1c} in GDM diagnostic strategy, which can reduce the number of pregnant women subjected to the GCT. Our results confirm that mean HbA_{1c} levels were lower in the normalgroup,similar to previous results [16, 24]. Although the difference in HbA_{1c} level between groups is smaller than the difference in the mean GCT value between groups (70.26 mg/dL; Table 1), these parameters reflect two different, although related, situations. The GCT value is an indicator of altered postprandial glycemia that begins during the third trimester in GDM, while HbA_{1c} level indicates an increase in average glycemia, which begins weeks before in GDM [15]. We believe that both parameters can be useful in GDM diagnostic strategies.

The incidence of GDM in our study population (5.4%, Table 1) is similar to the 5% GDM incidence reported for Andalucia, our region [17]. Thus, our population (and region) can be considered at low-risk for GDM. Universal screening based on the GCT or other OGTTs is excessive, especially in low-risk populations; the characteristics of each population should be studied and the diagnostic strategy should be adapted according to the incidence [3, 4, 12].

We propose a low threshold determined between the 24th and 28th gestational weeks as a screening test prior to the GCT. These low threshold results are promising; a simple, cheap, and non-aggressive test, such as for HbA_{1c}, could lead to a 14% reduction in the number of women subjected to the GCT, with no false negatives, or a 21% reduction with only two false negatives. The low performance at the high cut-off could be explained in part by the fact that our participants are from a low-risk population. Another important reason is that according to the protocol of our region, pregnant women undergo high-risk screening during the first trimester [20]. Thus, many high-risk pregnant women had already been detected before the 24th week when we recruited our population. In addition, of the 39 participants who would have avoided the GCT, 24 had a negative GCT result. However, it is important to remember that S of the GCT using 140 mg/dL as the cut-off is between 70% and 88% [6]; consequently, some of these negative GCT results could be false negatives.

Similar results were reported by one of the few studies that followed diagnostic criteria comparable to ours (two-step strategy, GCT+OGTT). Agarwal, *et al*. [16] analyzed a high-risk population from the United Arab Emirates following the Carpenter and Coustan criteria [21], which are more inclusive than those of the NDDG [12]. However, the results are quite similar (Table 3). A notable difference is that our study identified only two false negatives (in 607 participants) when using the mentioned cut-off, while Agarwal, *et al.* [16] reported nine false negatives (in 426 participants).

More recently, Kwon, *et al.* [22] published a similar study, also based on the Carpenter and Coustan criteria [21]; however, their population included 321 Korean women at risk of GDM (positive GCT). They proposed the use of HbA_{1c} as a screening test, with an AUC=0.824 and a cut-off point of 5.1%, with S=91.3%. However, they did not report any data regarding test performance that would allow us to compare it with our results.

In recent years, especially since the publication of Lowe, *et al.* [11], new studies proposing the use of HbA_{1c} as a biomarker of GDM have been emerging [15, 16]. These studies (diagnostic performance parameters are listed in Table 3) support our pro-

Table 3. Studies that propose the use of HbA_{1c} for the diagnosis of GDM

Study (country, year)	Criteria	AUC	Cut-off %HbA_{1c} (mmol/mol)	S (%)	SP (%)	PV (%)	LR
Renz, *et al.* [15] (Brazil, 2015)	IADPSG	0.76	<5.00 (31)	89.70	32.60	-	0.32
Agarwal, *et al.* [16] (United Arab Emirates, 2000)	CC	0.72	≥5.80 (40)	26.40	94.90	-	5.14
			≥6.50 (48)	-	98.1	71.4	6.84
Kwon, *et al.* [22] (Korea, 2015)	CC	0.82	<5.05 (32)	91.30	62	-	-
Khalafallah, *et al.* [23] (Australia, 2016)	ADIPS	-	<5.40 (36)	27	95	91.20	-
Ye, *et al.* [24] (China, 2016)	IADPSG	0.66	<4.80 (29)	85	31.80	87.80	-
			≥5.50 (37)	14.8	95.70	48	-

Abbreviations: HbA_{1c}, glycated Hb; GDM, gestational diabetes mellitus; AUC, area under the curve; S, sensitivity; SP, specificity; PV, predictive value (negative and positive); LR, likelihood ratio (negative and positive); CC, Carpenter and Coustan; IADPSG, International Association of Diabetes and Pregnancy Study Groups; ADIPS, Australian Diabetes in Pregnancy Society.

posal of HbA_{1c} as a useful tool in the diagnosis of GDM. All of these studies propose screening thresholds to be used in an attempt to reduce the number of GCTs or OGTTs by identifying GDM in high-risk populations or using the more inclusive IADPSG criteria [1]; thus, they used lower Sthan our study, which aimed at identifying GDM in low-risk women.

When comparing our work with the aforementioned ones, Ye, *et al.* [24] reported an AUC of 0.66, similar to our AUC (0.68); however, the value proposed by Renz, *et al.* [15] (AUC=0.757) is somewhat higher (Table 3).

The HbA_{1c} cut-off points chosen for all these studies, with the exception of Ye, *et al.* [24], are always higher than the levels in our population. This could be explained by ethnic differences in Hb glycosylation [25] but also because of our higher level S.

A possible limitation of our study is that it was carried out in the context of a two-step diagnostic strategy. This strategy follows national and international recommendations (Spanish Group of Diabetes and Pregnancy [14], ADA [4], ACOG [5]); however, a significant number of pregnant women in our population have been subjected to only a GCT. Asthe GCT has S between 70% and 88% with cut-off point of 140 mg/dL [6], some of these pregnant women could have had false negatives, a situation that we cannot control and is beyond the objectives of this study.

In a low-risk population, it is difficult to justify subjecting all pregnant women to a test as unpleasant and costly as the GCT. We provide an alternative for 14% of pregnant women, identified as at low risk of GDM, for whom the GCT could be avoided by measuring HbA_{1c}, a routine marker.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

ORCID

Jose-Maria Maesa https://orcid.org/0000-0001-5262-9108
Patricia Fernandez-Riejos https://orcid.org/0000-0001-9242-6006
Concepcion Gonzalez-Rodriguez https://orcid.org/0000-0001-6628-688X
Victor Sanchez-Margalet https://orcid.org/0000-0001-8638-8680

REFERENCES

1. International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010;33:676-82.
2. HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U. Hyperglycemia and adverse pegnancy-outcomes. New Engl J Med 2008;358:1991-2002.
3. Farrar D, Duley L, Medley N, Lawlor DA. Different strategies for diagnosing gestational diabetes to improve maternal and infant health. Cochrane Database Syst Rev 2015;1:CD007122.
4. American Diabetes Association. Management of diabetes in pregnancy: standards of medical care in diabetes - 2019. Diabetes Care 2019;42(S1): S165-72.
5. Committee on Practice Bulletins - Obstetrics. Practice Bulletin No. 137: Gestational diabetes mellitus. ObstetGynecol 2013;122:406-16.
6. Donovan L, Hartling L, Muise M, Guthrie A, Vandermeer B, Dryden DM. Screening tests for gestational diabetes: a systematic review for the US Preventive Services Task Force. Ann Intern Med 2013;159:115-22.
7. Bonongwe P, Lindow SW, Coetzee EJ. Reproducibility of a 75G oral glucose tolerance test in pregnant women. J Perinat Med 2015;43:333-8.
8. Maesa JM, Fernández-Riejos P, Mora CS, de Toro M, Valladares PM, González-Rodriguez C. Evaluation of Bio-Rad D-100 HbA1c analyzer against Tosoh G8 and Menarini HA-8180V. Pract Lab Med 2016;5:57-64.
9. WHO. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. 2011;1-25.
10. International Expert Committee. International expert committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32:1327-34.
11. Lowe LP, Metzger BE, Dyer AR, Lowe J, McCance DR, Lappin TR, et al. Hyperglycemia and adverse pregnancy outcome (HAPO) Study: associations of maternal A1C and glucose with pregnancy outcomes. Diabetes Care 2012;35:574-80.
12. Maesa JM, Fernandez-Riejos P, Sanchez-Mora C, Toro-Crespo M, Gonzalez-Rodriguez C. Application of six sigma model to evaluate the analytical quality of four HbA1c analyzers.Clin Lab 2017;63:79-83.
13. GrupoEspañol Diabetes y Embarazo. Asistencia a la gestante con diabetes. Guía de prácticaclínicaactualizadaen 2014. Diabetologia 2015; 31:45-59.
14. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28: 1039-57.
15. Renz PB, Cavagnolli G, Weinert LS, Silveiro SP, Camargo JL. HbA1c test as a tool in the diagnosis of gestational diabetes mellitus. PLoS One 2015; 10:e0135989.
16. Agarwal MM, Hughes PF, Punnose J, Ezimokhai M, Thomas L. Gestational diabetes screening of a multiethnic, high-risk population using glycated proteins. Diabetes Res ClinPract 2001;51:67-73.
17. Plan integral de diabetes en Andalucía. Análisissituación 2013. https://www.juntadeandalucia.es/export/drupaljda/salud_5af065335c108_pidma3.pdf (Updated on 2013).
18. Benhalima K, Damm P, Van Assche A, Mathieu C, Devlieger R, Mahmood T, et al. Screening for gestational diabetes in Europe: where do we stand and how to move forward:A scientific paper commissioned by the European Board & College of Obstetrics and Gynaecology (EBCOG). Eur J ObstetGynecolReprodBiol 2016;201:192-6.
19. National Institute for Health and Care Excellence. Diabetes in pregnancy: management of diabetes and its complications from preconception to the postnatal period. 2015;2-65.
20. Aceituno Velasco L, Aguado Maldonado J, Arribas Mir L, Caño Aguilar A,

Corona Páez I, Martín López J, et al. Procesoasistencialintegradoembarazo, parto y puerperio. Junta Andalucía ConserjeríaIgualdad, Salud u PolíticasSoc 2014;3:1-73.
21. Carpenter MW and Coustan DR. Criteria for screening tests for gestational diabetes. Am J ObstetGynecol 1982;144:768-73.
22. Kwon SS, Kwon JY, Park YW, Kim YH, Lim JB. HbA1c for diagnosis and prognosis of gestational diabetes mellitus. Diabetes Res ClinPract 2015;110:38-43.
23. Khalafallah A, Phuah E, Al-Barazan AM, Nikakis I, Radford A, Clarkson W, et al. Glycosylated haemoglobin for screening and diagnosis of gestational diabetes mellitus. BMJ Open 2016;6:e011059.
24. Ye M, Liu Y, Cao X, Yao F, Liu B, Li Y, et al. The utility of HbA1c for screening gestational diabetes mellitus and its relationship with adverse pregnancy outcomes. Diabetes Res ClinPract 2016;114:43-9.
25. Wolfenbuttel BH, Herman WH, Gross JL, Dharmalingam M, Honghua HJ, Hardin DS. Ethnic differences in glycemic markers in patients with type 2 diabetes. Diabetes Care 2013;36:2931-6.