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Glycosylated Haemoglobin A1c for screening and diagnosis of gestational diabetes mellitus

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ABSTRACT

Objectives

The oral glucose tolerance test (OGTT) is a cumbersome test that is time consuming, labour intensive and often poorly tolerated by pregnant women. To date, glycosylated Hb or HbA1c, is the most accepted measure of chronic glycaemia outside of pregnancy. HbA1c is an uncomplicated test, less time consuming, does not require any specific patient preparation and is considered straightforward compared to the OGTT.

Therefore, we tested prospectively the utility of the HbA1c when used as a screening tool in pregnancy for gestational diabetes mellitus (GDM).

Settings: Primary Health Care. Single tertiary referral centre, Tasmania, Australia.

Participants

A direct comparison between HbA1c levels and the OGTT results in pregnant women, tested concurrently at the 24-28 gestational week, was undertaken. A full profile of 480 pregnant women during the period from September 2012 till July 2014 was completed. Median and mean age of participants was 29 years (range, 18-47 years).

Interventions

A simultaneous prospective assessment of HbA1c versus standard OGTT in a cohort of consecutive pregnant women presenting to our institute was performed.

Results

The number of women who had GDM according to the OGTT study criteria was 57, representing 11.88% of the evaluated 480 pregnant women. Using a cut-off value for HbA1c at 5.1% (32mmol/mol) for detecting GDM showed sensitivity of 61% and specificity of 68% with negative predictive value (NPV) of 93%, versus sensitivity of 27% and specificity of 98% with NPV of 91% when using HbA1c cut-off value of >5.4% (36mmol/mol).

Conclusions

Our results suggest that pregnant women with an HbA1c of >5.4%(36mmol/mol) should proceed with an OGTT. This may result in a significant reduction in the burden of testing on both patients and testing facility staff and resources. Further investigations are required to integrate and optimise the HbA1c as a single non-fasting screening tool for GDM.

Key words: HbA1c, glucose tolerance test, pregnancy, gestational diabetes, screening

The study was registered prospectively in the Australian New Zealand Clinical Trials Registry as a part of www.anzctr.org.au/ACTRN12611000739910.aspx trial.

Article summary

'Strengths and limitations of this study'

1. Oral glucose tolerance test (OGTT) is a standard screening test for gestational diabetes mellitus (GDM), however it requires fasting and 3 separate blood tests over more than 2 hours, is often poorly tolerated by pregnant women and is labour-intensive, adding an additional burden to an overstretched health system.
2. HbA1c is a simple single non-fasting test that may give insight in gestational diabetes.
3. Our study of 480 pregnant women suggests that HbA1c could be a useful screening tool for GDM.
4. HbA1c is safe, cost effective and more convenient for pregnant women.
5. Few patients would miss the diagnosis of GDM by using this screening tool.

INTRODUCTION

Pregnancies affected by gestational diabetes mellitus (GDM) are at risk of developing a number of serious obstetric complications such as foetal growth abnormalities, shoulder dystocia, birth injury, prematurity and increased Caesarean section rate,¹ as well as having long term implications for the wellbeing of mother and infant. The risk of adverse perinatal and maternal outcomes is directly proportional to the degrees of hyperglycaemia, with linear relationship between maternal glucose and various neonatal outcomes.

The current screening process using the revised Australasian Diabetes in Pregnancy Society (ADIPS) guidelines 2012 (IADPSG criteria) has resulted in an increase in the detected incidence of GDM in the Australian population from 6-8% to 13%.² The guidelines recommend a 75g oral glucose tolerance test (OGTT) at 26-28 weeks for all pregnant women.

However, the OGTT is a cumbersome test that is time consuming, labour intensive and often poorly tolerated by pregnant women. The patient must be fasted, sit for over 2 hours and have at least 3 venepunctures. The gravida is prone to nausea and vomiting from delayed gastric emptying. This, coupled with gestational oedema compromising venous access, can lead to an invalid test result. Furthermore, the recommendation for universal screening has increased the burden of testing.

The instability of blood glucose *ex vivo* leads to significant inter-laboratory variation of results. It is thought to vary by up to 14% in a third of cases.³

Whilst guidelines are in place, the glucose threshold values for diagnosis and methods of testing for GDM vary greatly from one institution to another. Moreover, as it is a specialised test, many collection centres do not provide this service, particularly in rural and remote locations, potentially disadvantaging an already vulnerable cohort of women.⁴

The need for a simpler, more universally acceptable and accessible test is becoming increasingly apparent.

Glycosylated Hb, or HbA1c, is currently the most accepted measure of chronic glycaemia outside of pregnancy.

The National Health and Medical Research Council (NHMRC) guidelines 2009 recommend HbA1c to be the basis for diagnosis of Type 2 Diabetes Mellitus, with a value of 48 mmol/L or 6.5% or greater being confirmatory.⁵

HbA1c is the product of an irreversible non-enzymatic binding of glucose to plasma proteins, specifically haemoglobin. The mean plasma glucose over the erythrocyte life span is correlated with degree of glycosylation. It is a single non-fasting blood test and reflects glucose levels over the previous 4-8 weeks. As compared to glucose testing it has been shown to have greater reliability with <6% inter-laboratory variation.³

Further comparisons with fasting blood glucose and 2 hour post prandial glucose have shown HbA1c to have a more precise value within subject biological variability,⁶ as it does not appear to be affected by diurnal variation, meals, fasting, acute stress or by the large number of common drugs known to influence glucose metabolism.⁷

The test is validated for a red cell survival time of approximately 3 months. Therefore, results need to be interpreted carefully in the clinical situation whereby erythrocyte half-life is significantly shortened by, for example, haemoglobinopathies, haemolysis, transfusion, anaemia and chronic renal failure.

Dilutional anaemia of pregnancy and increased erythrocyte turnover have to date hampered its acceptance as a tool for screening, if not diagnosis, of GDM.⁸

The accuracy of HbA1c as a screening test in pregnancy has been extensively studied over the last three decades and results have been inconsistent.⁹⁻¹³

Many of these studies were conducted prior to the Hyperglycemia and Adverse Pregnancy Outcome (HAPO 2008) findings,¹⁴ upon which the current International Association of Diabetes and Pregnancy Study Groups (IADPSG 2010)¹⁵ and ADIPS² screening strategies are based. Consequently, there is significant heterogeneity in both

methods used for screening for GDM and diagnostic glucose thresholds when comparing with HbA1c. In addition, and importantly, many studies have used the same reference range for HbA1c in both pregnant and non-pregnant patients. Nonetheless, the results of these studies have been inconclusive. The overlap of HbA1c values between normal and GDM affected pregnancies has always been too great for HbA1c to have sufficient sensitivity and specificity to meet the screening requirements of a test.

Using current screening guidelines, Neilsen *et al* 2004 have shown the normal upper range of HbA1c in early pregnancy to be significantly lower than levels found outside of pregnancy, (5.7%/39 mmol/mol) and did not significantly differ from late pregnancy (5.6%/38 mmol/mol).¹⁶

This study looked at non-GDM women at 14 weeks and 33 weeks as determined by negative OGTT values. Haemoglobin levels, however, were not accounted for and anaemia could have inadvertently lowered results.

O'Connor *et al* 2012 did find trimester specific reference intervals for HbA1c in a study of 246 non-diabetic pregnant women with normal haemoglobin levels. First trimester: 4.8-5.5% (29-37mmol/mol), second trimester: 4.4-5.4% (25-36 mmol/mol) and third trimester: 4.4-5.4% (25-36 mmol/mol).¹⁷

Objectives of the study:

The aim of the study is to provide an objective assessment of the utility of HbA1c when used as a screening tool in pregnancy. A direct comparison of HbA1c levels with results of the OGTT in gravid women, tested concurrently at the 24-28 gestational week, was undertaken.

PATIENTS AND METHODS

Participants

We recruited 480 pregnant women during the period from September 2012 until July 2014. For these patients, we performed a simultaneous prospective assessment of HbA1c versus standard OGTT in a cohort of consecutive pregnant women presenting to the Launceston General Hospital (a tertiary referral teaching hospital in Tasmania, Australia). Pregnant women were approached when attend their routine third trimester OGTT test at our institution. Written informed consent was obtained from all subjects. For these patients, simultaneous Full Blood Count (FBC) and Iron studies were performed as per our routine antenatal assessment. Median and mean age of participants was 29 years (range, 18-47).

The trial was approved by the Tasmanian Human Research Ethics Committee as a part of another trial targeting the same population studying thyroid disease of pregnancy and was registered in the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au/ACTRN12611000739910.aspx) and the World Health Organization Clinical Trials Registry (www.who.int/trialsearch/Trial2.aspx?trialid=ACTRN12611000739910).

All patients were tested in the third trimester of pregnancy, according to our policy at the LGH for OGTT estimation at the time of study, with a median of 26 weeks and mean of 25.7 weeks' gestation. Twin pregnancies were excluded. Women having OGTT at 26 weeks did not have an earlier diagnosis of GDM.

Parameters such as gravity, parity, ethnicity, personal and family history of diabetes, type of delivery, complications of pregnancy, body mass index and perinatal data, as well as data regarding the infants' APGAR scores, weight and sex were collected prospectively.

HbA1c was measured by immunoassay using the DCA 2000 (Siemens Ltd., Marburg, Germany). The DCA 2000 analyser measures HbA1c standardised to the National Glycohemoglobin Standardization Program (NGSP), which is in turn aligned to the Diabetes Control and Complications Trial (DCCT) results with international standardization as set by the International Federation of Clinical Chemistry (IFCC) (<http://www.ngsp.org/certified.asp>).

Glucose tolerance was measured at the same time using the Abbott glucose hexokinase method on an Architect C8000 analyser (Abbott Australasia Pty. Ltd.).

For the purpose of our study, GDM is defined as present if fasting blood glucose is ≥ 5.1 mmol/L or GTT 1hr ≥ 10.0 mmol/L OR GTT 2 hr ≥ 8.5 mmol/L. GDM is defined as not present if fasting blood glucose < 5.1 mmol/L and GTT 1hr < 10 mmol/L and GTT 2 hr < 8.5 mmol/L.

STATISTICAL ANALYSIS

Patient characteristics were expressed as percentages for categorical variables, and as mean and standard deviation, or median with interquartile range for continuous variables.

Groups were compared using *t*-test, and multiple-category variables were analysed using analysis of variance.

Bland-Altman plot was used to assess the agreement between HbA1c levels and the values of the glucose tolerance test. Receiver operating characteristic (ROC) curve was performed to assess the discriminative capacity of HbA1c for detection of GDM. Sensitivity, specificity, predictive values, false positive, and negative rates were calculated. All analyses were performed with SAS (version 9.3).

RESULTS

A number of variables were studied in the 480 consecutive pregnant women who presented for routine assessment at the LGH. Approximately 12% of pregnant women were diagnosed with GDM according to the ADIPS criteria and this is consistent with the expected incidence of GDM in Australia.

The mean gestational age at enrolment was 25.7 weeks (SD 3.3) with a mean fasting glucose level of 4.37 mmol/L (SD 0.46), 6.85 mmol/L (SD 1.7) at 1 hour and 5.84 mmol/L (SD 1.45) at 2 hours. The mean HbA1c was 4.8 % (29mmol/L) (SD 0.36). Full blood count (FBC) and iron studies were assessed for the same subjects, showing mean Hb of 119 g/L (SD 8.71) and mean ferritin of 20 mcg/L (SD 60).

A Spearman correlation between OGTT and HbA1c showed significant results for 1h and 2h OGTT with HbA1c ($p < 0.0001$) (Table 1). However, no such correlations

existed for Hb and OGTT ($p=0.38$ and 0.25 respectively). Further analyses demonstrated that ferritin and Hb levels correlate to HbA1c ($p=0.02$, $p<0.0001$ respectively) (Table 1). Bland-Altman correlations between OGTT and HbA1c are shown in Figures 1 and 2.

Table 1: Spearman correlations between OGTT and HbA1c

		Spearman Correlation Coefficients				
		Prob > r under H0: Rho=0				
		Number of Observations				
	Fasting Blood Sugar	OGTT (mmol/L) 1 HR	OGTT (mmol/L) 2ND	HbA1c %	Ferritin	Hb
Fasting blood glucose	1.00000					
OGTT (mmol/L)/1Hr	0.36109 <.0001 475	1.00000				
OGTT (mmol/L) 2 Hr	0.31745 <.0001 480	0.61037 <.0001 475	1.00000			
HbA1c %	0.34298 <.0001 438	0.21911 <.0001 434	0.22906 <.0001 438	1.00000		
Ferritin	0.03316 0.4757 465	0.11589 0.0129 460	0.00636 0.8912 465	-0.10974 0.0233 427	1.00000	
Hb	-0.03201 0.4860 476	0.03996 0.3869 471	-0.05220 0.2557 476	-0.26569 <.0001 436	0.23075 <.0001 463	1.00000

The number of women who had gestational diabetes according to the OGTT study criteria was 57 in our cohort, representing 11.88% of the 480 studied pregnant women. In the same trial period at our laboratory there were 97 out of 1795 pregnant women tested (5.4%) who had a fasting blood glucose level >5.1 mmol/L while at 2 h the glucose level was >8.5 mmol/L in 96 out of 1775 patients (5%) in the OGTT.

It is worth noting that most of the patients studied (88%) were iron deficient with ferritin level <30 mcg/L, while 57% had ferritin <15 mcg/L. The HbA1c distribution by GDM status is shown in Figure 3.

Using an arbitrary cut-off value for HbA1c at 5.1% (32 mmol/mol) to detect GDM showed sensitivity of 61% and specificity of 68% with negative predictive value (NPV) of 93% versus sensitivity of 27% and specificity of 98% with NPV of 91% when using a HbA1c cut-off value of >5.4% (36 mmol/mol) (Table 2).

Table 2: Sensitivity and Specificity for all values of HbA1c

HbA1c %	Sensitivity	Specificity	PPV	NPV
10	0	0.997	0	0.888
6.1	0.02	0.997	0.5	0.89
6	0.041	0.997	0.667	0.892
5.9	0.061	0.997	0.75	0.894
5.8	0.082	0.997	0.8	0.896
5.7	0.102	0.995	0.714	0.898
5.6	0.122	0.99	0.6	0.9
5.5	0.224	0.982	0.611	0.91
5.4	0.265	0.954	0.419	0.912
5.3	0.347	0.884	0.274	0.915
5.2	0.551	0.797	0.255	0.934
5.1	0.612	0.676	0.192	0.933
5	0.694	0.519	0.154	0.931
4.9	0.735	0.314	0.119	0.904
4.8	0.816	0.18	0.111	0.886
4.7	0.959	0.1	0.118	0.951
4.6	0.959	0.046	0.112	0.9

PPV=positive predictive value; NPV=negative predictive value

Regarding ethnicity, 93% of the population studied were Caucasian, with 4% Asian and 3% Aboriginal. There was no significant correlation between GDM or HbA1c and ethnicity. Nevertheless this study is not statistically powered to detect such differences.

The sex of the baby, complications of pregnancy or type of delivery did not show a correlation to HbA1c levels. However, GDM itself was associated with more

complications during pregnancy ($p < 0.0001$) compared to non-GDM pregnant women but did not influence intra or postpartum or natal complications (Table 3).

Table 3: Does GDM status influence complications during pregnancy, intrapartum postpartum?

		GDM status (N, %)		P value*
		0= No gestational diabetes	1= Gestational diabetes	
Complications During Pregnancy	1 = No	305 (97)	8 (3)	<.0001
	2 = Yes	95 (68)	45 (32)	-
	3 = Insufficient information	21 (91)	2 (9)	-
Intrapartum complications	1 = No	378 (88)	52 (12)	0.8203
	2 = Yes	20 (87)	3 (13)	-
	3 = Insufficient information	23 (92)	2 (8)	-
Postpartum complications;	1 = No	362 (88)	50 (12)	0.9047
	2 = Yes	36 (88)	5 (12)	-
	3 = Insufficient information	23 (92)	2 (8)	-

*Groups were compared with Chi-Square or Fisher tests.

Proposed cost saving

The use of HbA_{1c} in the context of gestational diabetes screening provides an economy at the point of specimen collection. The resource requirement for the specimen collection facility may be significantly reduced. For example, bleeding a patient once for an HbA_{1c} as opposed to three times for a GTT (Baseline, 1 hour and 2 hour). Consumables and equipment, salaries and accommodation are also reduced which may, over time, support an economic argument.

Although a cost analysis was not the primary objective of the study, it is worthwhile attempting to calculate approximate costs of Consumables/Equipment/Accommodation per bleed. At the LGH public laboratory the laboratory cost per bleed is approximately \$2 USD and the salary of the nurse performing the venepuncture, is approximately \$10 per bleed. The cost of testing an additional blood sample is approximately \$9 per given episode.

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This could lead to total savings in the order of \$33 per episode. Scaled up at 50 per week for a medium sized laboratory, for example, could yield a saving of approximately \$85,800 per year.

Furthermore, in health systems that often have resourcing issues, such as staff and accommodation, it is far more efficient to have a patient bled once rather than three times and accommodate them (and their party) in the waiting room for more than two hours.

In addition, there is an economy for the patients themselves in terms of time off work, parking and issues such as child care when applicable.

DISCUSSION

Since the time of the original publications investigating the value of HbA1c for GDM diagnosis, laboratory testing of HbA1c has become increasingly standardised and evolved to be a simpler, more accurate and automated test. It has developed from simple ELISA to turbidometric inhibition immunoassay (TINIA), an assay method largely unaffected by either haemoglobinopathies or uraemia, through to precision liquid chromatography. Previously, HbA1c results could not be compared from one laboratory to another let alone one country to another.¹⁸ However, since international standardisation of the assay, this is no longer the case.

A meta-analysis of 43 studies, involving over 2812 GDM patients in China compared to 5918 controls concluded that, based on the Summary Receiver Operating Characteristic (SROC) curve analysis, HbA1c is a useful diagnostic tool for confirming GDM.¹⁹ Studies from 2001 to 2012 were included in this meta-analysis with varying diagnostic criteria for GDM and cut off values for HbA1c, such that the authors recommended HbA1c to be tested in parallel with conventional tests.

Rajput *et al* 2012 studied 607 women between 24-28 weeks' gestation. They were evaluated for GDM using OGTT based on ADA criteria (2hr 75g OGTT or "one step strategy") and concurrently tested for HbA1c.²⁰ A cut-off value of $\geq 5.4\%$ (36 mmol/mol) had a sensitivity of 85.7% and specificity of 61.1%. Only 2.8% would

have been diagnosed incorrectly as GDM and reporters state that it would have obviated the need for OGTT in 61.8% of cases.

In a smaller retrospective study of 145 high risk Saudi Arabian women, Aldasouqi *et al* in 2008 demonstrated the use of HbA1c in detecting 87% of the GDM patients diagnosed on OGTT, missing 12%. However, in this study the cutoff HbA1c was 6% (42 mmol/mol).²¹

Belaji *et al* 2007 determined that the normal mean HbA1c values in Asian Indian women ranged between 5.36% +/- 0.36% and $\geq 6\%$ (42 mmol/mol) in women with GDM in a study of 507 women. This study was interesting in that all trimesters were studied. Women who had a positive HbA1c but negative OGTT in the first trimester subsequently developed GDM.²²

The implication of this is twofold. First, the HbA1c false positives identified in other studies may have actually been true positives with OGTT results being false negatives. Second, if the results of this study are reproducible, then management instituted earlier may have an impact on outcome for babies of mothers who, despite being screened, detected and treated during third trimester, still suffer the consequences of GDM.

Genetically determined variations in the degree of glycosylation of haemoglobin, independent of glycaemia, are thought to exist and are reflected in ethnic differences in HbA1c levels.⁸ This suggests that population reference ranges need to be established prior to universally implementing HbA1c as a screening test in gestational diabetes. It is worth noting that the vast majority of our studied population were Caucasian (94%).

The use of HbA1c as a screening tool for gestational diabetes has yet to be evaluated in an Australian population. To our knowledge, there is no published data comparing the 75g 2 step OGTT as per current guidelines with HbA1c in establishing the diagnosis of GDM. It is anticipated that validating the diagnostic utility of HbA1c in pregnancy in Australia would result in a reduction in the burden of testing, increase

patient access and compliance, therefore facilitate the management of the gestational diabetic mother and perhaps improves perinatal and maternal outcomes.

We found that the application of HbA1c as a method for screening GDM within the Australian population needs further refining and definition for its use. Outside of pregnancy, it has been established that HbA1c measurement can accelerate and facilitate patient screening, diagnosis and management of diabetes. The usual cut-off value for diagnosing diabetes outside pregnancy seems to be much higher than the cut-off value needed to diagnose diabetes associated with pregnancy. This is in concordance with other studies already detailed.

The results of our study indicate that, using an HbA1c level of 5.4% (36 mmol/mol) at third trimester ((26 week) has a specificity of 98%, sensitivity of 27%, and NPV of 91% in detecting GDM, in line with the reference range for pregnancy reported by other studies.^{17,20} Using a cut-off value of HbA1c 5.1% (32 mmol/mol), the sensitivity increased to 55%, at the expense of specificity, which decreased to 80%. The positive association between HbA1c and OGTT is an advantage, but the low sensitivity of HbA1c becomes a hurdle in standardising such a test in pregnancy.

Further investigations are required to integrate HbA1c as a single, non-fasting diagnostic test for GDM. However, the high NPV may make it useful as an initial screening test. For example, patients with an HbA1c >5.4% (36 mmol/mol) should proceed with an OGTT. This alone would generate a significant reduction in the burden of testing.

It is worth noting the additional disadvantages of OGTT potentially avoided by the use of HbA1c. These include the need to fast, non-toleration of glucose ingestion, nausea, a more than 2 hour stay in the laboratory, multiple venpunctures, associated stress and discomfort, greater use of consumables and finally increased time requirements on the blood nurse and the associated costs.

Our study has a few shortcomings due to the lack of sensitivity of HbA1c and that some patients, although a small number, will miss the diagnosis of GDM. Therefore, further optimisation of the test is required prior to its application as a screening tool

for GDM. It is likely that the pathophysiology of GDM is different from DM in the general population. However, GDM may be an indicator of increased risk of non-insulin dependent type 2 DM in the postnatal period. Pregnancy is a state of diabetogenesis. Hormones secreted by the placenta, including growth hormone, corticotropin releasing hormone, placental lactogen and progesterone, all act to increase insulin resistance in the mother, serving to ensure adequate supply of nutrients to the developing foetus.²³ Where the mother has insufficient pancreatic function to cope with this increasing insulin resistance, diabetes ensues.

In contrast, in Type 2 (non-pregnant) diabetes mellitus, increasing insulin resistance obviously is not mediated by a placenta but rather by a complex interplay between genetic predisposition, obesity and decreased physical activity. Abdominal fat is metabolically active, producing hormones that promote insulin resistance. Leptin, tumor necrosis factor, alpha and resistin are among the many “adipokines” implicated in insulin resistance and subsequent development of Type 2 DM.²⁴⁻²⁶ Adipose cells, furthermore, are thought to trigger chronic inflammation, which in turn contributes to the development of insulin resistance.²⁷

However, the difference in the performance of HbA1c in non-pregnant versus pregnant populations may not be entirely explained by different pathophysiology.

Two randomised trials have shown that even treatment of mild gestational diabetes improves outcome and provides opportunity to prevent macrosomia, preeclampsia and birth trauma.^{28,29} These studies have driven the lowered threshold for diagnosing GDM.

In summary, employing a cut-off value for HbA1c at >5.4 % (36 mmol/mol) for detecting GDM showed NPV of 91% and specificity of 98%. The high specificity and NPV value may be useful as an initial screening test. Our results suggest that patients with a HbA1c of >5.4% (36 mmol/mol) should proceed with an OGTT. This may result in significant reduction in the burden of testing on both patients and testing facility, staff and resources. Further investigations are required to integrate HbA1c as a single non-fasting screening tool for GDM with optimisation of the cut-off value.

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REFERENCES

1. Casey BM, Lucas MJ, McIntire DD, Leveno KJ. Pregnancy outcomes in women with gestational diabetes compared with the general obstetric population. *Obstet Gynecol.* 1997;90:869-73
2. Nankervis A, McIntyre H, Moses R, Ross G, Callaway L, Porter C, et al. Australasian Diabetes in Pregnancy Society (ADIPS) consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia. 2012.
3. d'Emden M. Glycated haemoglobin for the diagnosis of diabetes. *Australian Prescriber.* 2014;37:98-100
4. Hoang H, Le Q. Comprehensive picture of rural women's needs in maternity care in Tasmania, Australia. *Aust J Rural Health.* 2013;21:197-202
5. Colaguri S D, D, Girgis S et al. National evidence based guideline for case detection and diagnosis of type 2 diabetes. Diabetes Australia and National Health and Medical Research Council; Canberra2009.
6. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med.* 2007;167:1545-51
7. Pandit MK, Burke J, Gustafson AB, Minocha A, Peiris AN. Drug-induced disorders of glucose tolerance. *Ann Intern Med.* 1993;118:529-39
8. Lippi G, Targher G. Glycated hemoglobin (HbA1c): old dogmas, a new perspective? *Clin Chem Lab Med.* 2010;48:609-14
9. Agarwal MM, Hughes PF, Punnonen J, Ezimokhai M, Thomas L. Gestational diabetes screening of a multiethnic, high-risk population using glycated proteins. *Diabetes Res Clin Pract.* 2001;51:67-73
10. Agarwal MM, Dhatt GS, Punnonen J, Koster G. Gestational diabetes: a reappraisal of HbA1c as a screening test. *Acta Obstet Gynecol Scand.* 2005;84:1159-63
11. Pollak A, Brehm R, Havelec L, Lubec G, Malamitsi-Puchner A, Simbrunner G, et al. Total glycosylated hemoglobin in mothers of large-for-gestational-age

infants: a postpartum test for undetected maternal diabetes? *Biol Neonate*. 1981;40:129-35

12. Griffiths RJ, Vinall PS, Stickland MH, Wales JK. Haemoglobin A1c levels in normal and diabetic pregnancies. *Eur J Obstet Gynecol Reprod Biol*. 1987;24:195-200

13. Moses RG. HbA1c and the diagnosis of gestational diabetes mellitus--a test whose time has not yet come. *Diabetes Res Clin Pract*. 2012;98:3-4

14. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;358:1991-2002

15. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, 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

16. Nielsen LR, Ekbom P, Damm P, Glumer C, Frandsen MM, Jensen DM, et al. HbA1c levels are significantly lower in early and late pregnancy. *Diabetes Care*. 2004;27:1200-1

17. O'Connor C, O'Shea PM, Owens LA, Carmody L, Avalos G, Nestor L, et al. Trimester-specific reference intervals for haemoglobin A1c (HbA1c) in pregnancy. *Clin Chem Lab Med*. 2012;50:905-9

18. Gillery P. A history of HbA1c through Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2013;51:65-74

19. Tian QW, Xuan C, Wang HW, Zhao JX, Yu WL, Gao G, et al. Diagnostic accuracy of glycosylated hemoglobin in chinese patients with gestational diabetes mellitus: a meta-analysis based on 2,812 patients and 5,918 controls. *Genet Test Mol Biomarkers*. 2013;17:687-95

20. Rajput R, Yogesh Y, Rajput M, Nanda S. Utility of HbA1c for diagnosis of gestational diabetes mellitus. *Diabetes Res Clin Pract*. 2012;98:104-7

21. Aldasouqi SA, Solomon DJ, Bokhari SA, Khan PM, Muneera S, Gossain VV. Glycohemoglobin A1c: A promising screening tool in gestational diabetes mellitus. *Int J Diabetes Dev Ctries*. 2008;28:121-4

22. Balaji V, Madhuri BS, Ashalatha S, Sheela S, Suresh S, Seshiah V. A1C in gestational diabetes mellitus in Asian Indian women. *Diabetes Care*. 2007;30:1865-7
23. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr*. 2000;71:1256S-61S
24. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2009;302:179-88
25. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science*. 1993;259:87-91
26. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature*. 2001;409:307-12
27. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med*. 2011;17:179-88
28. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of Treatment of Gestational Diabetes Mellitus on Pregnancy Outcomes. *New England Journal of Medicine*. 2005;352:2477-86
29. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med*. 2009;361:1339-48

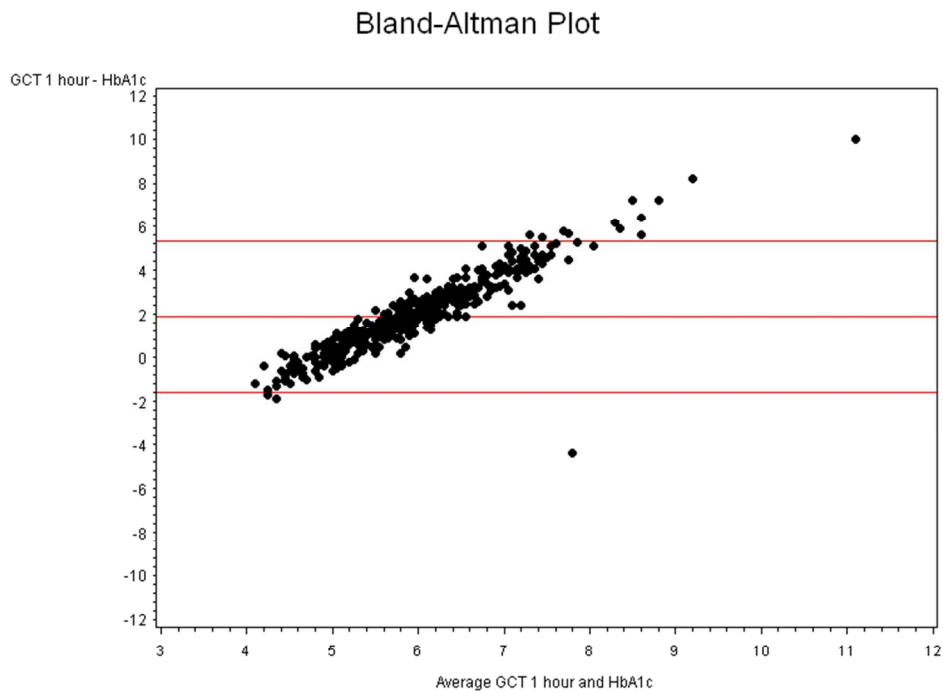
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Figure 1. Legend
Bland-Altman plot, OGTT 1 hour vs HbA1c

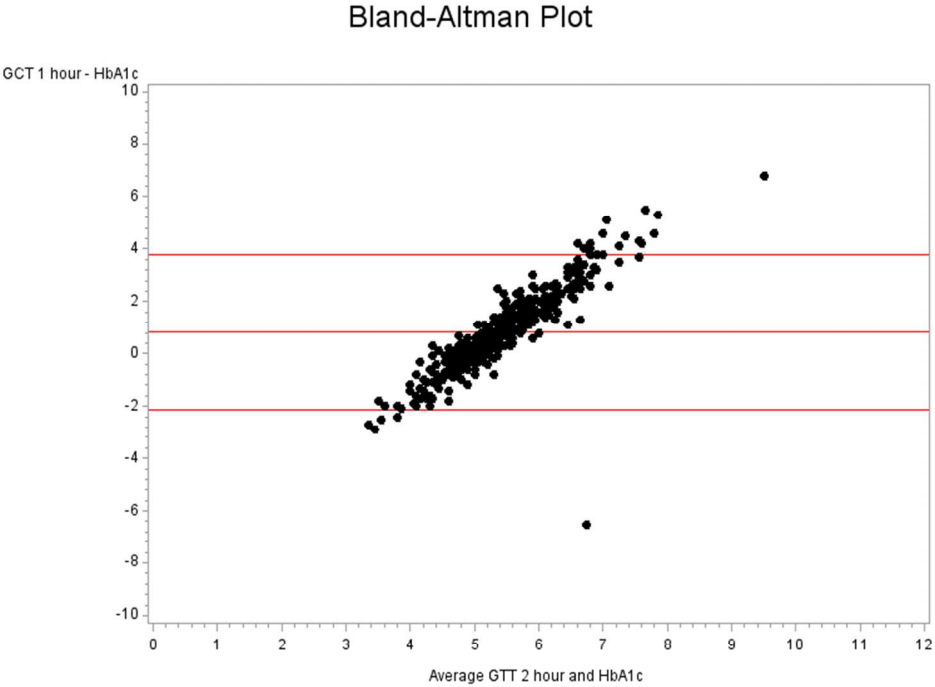
Figure 2. Legend
Bland-Altman plot, OGTT 2 hour vs HbA1c

Figure 3. Legend
HbA1c distribution by GDM status

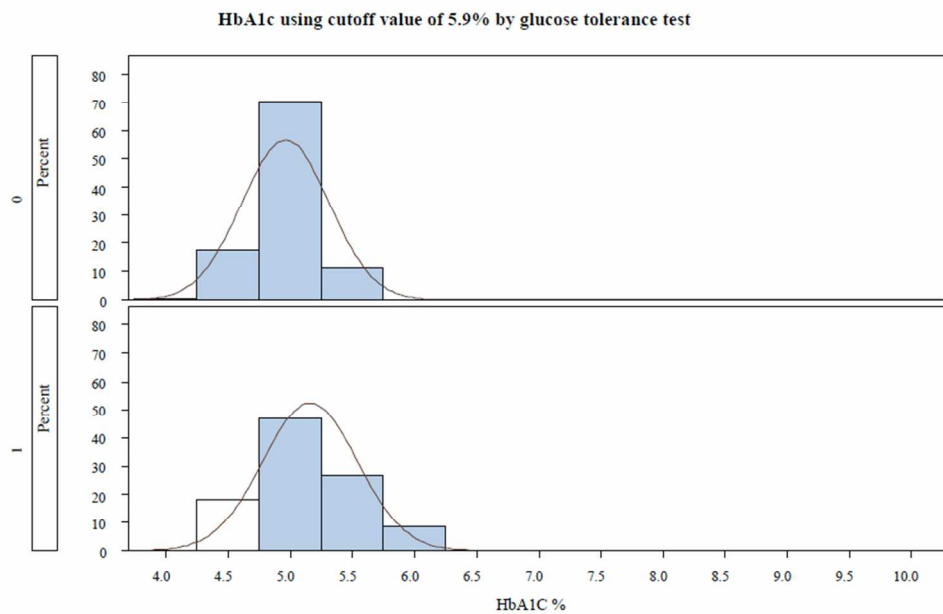
For peer review only



Bland-Altman plot, OGTT 1 hour vs HbA1c
281x211mm (72 x 72 DPI)



Bland-Altman plot, OGTT 2 hour vs HbA1c
282x211mm (72 x 72 DPI)



HbA1c distribution by GDM status
322x200mm (72 x 72 DPI)

Section & Topic	No	Item
TITLE OR ABSTRACT		Glycosylated Haemoglobin A1c for screening and diagnosis of gestational diabetes
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy sensitivity, specificity, predictive values
ABSTRACT		
	2	Structured summary of study design, methods, results, and conclusions; page 2
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test; page 5
	4	Study objectives and hypotheses; page 6
METHODS		
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	7	On what basis potentially eligible participants were identified; page 7 (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates); page 7
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	10b	Reference standard, in sufficient detail to allow replication; page 4
	11	Rationale for choosing the reference standard (if alternatives exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory; page 5
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory; not applicable
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test; page 8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard; page 8
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy; page 9
	15	How indeterminate index test or reference standard results were handled; page 10
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RESULTS		
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	29	Where the full study protocol can be accessed; page 7
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Glycosylated Haemoglobin A1c for screening and diagnosis of gestational diabetes mellitus

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Glycosylated Haemoglobin A1c for screening and diagnosis of gestational diabetes mellitus

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ABSTRACT

Objectives

The oral glucose tolerance test (OGTT) is a cumbersome test that is time consuming, labour intensive and often poorly tolerated by pregnant women. To date, glycosylated haemoglobin (HbA1c) is the most accepted measure of chronic glycaemia outside of pregnancy. HbA1c is an uncomplicated test, less time consuming, does not require any specific patient preparation and is considered straightforward compared to the OGTT. Therefore, we prospectively tested the utility of the HbA1c when used as a screening tool in pregnancy for gestational diabetes mellitus (GDM).

Settings: Primary Health Care. Single tertiary referral centre, Tasmania, Australia.

Participants

A direct comparison between HbA1c levels and the OGTT results in pregnant women, tested concurrently at the 24-28 gestational week, was undertaken. A full profile of 480 pregnant women during the period from September 2012 to July 2014 was completed. Median and mean age of participants was 29 years (range, 18-47 years).

Interventions

A simultaneous prospective assessment of HbA1c versus standard OGTT in a cohort of consecutive pregnant women presenting to our institute was performed.

Results

The number of women who had GDM according to OGTT criteria was 57, representing 11.9% of the evaluated 480 pregnant women. Using a cut-off value for HbA1c at 5.1% (32 mmol/mol) for detecting GDM showed sensitivity of 61% and specificity of 68% with negative predictive value (NPV) of 93%, versus sensitivity of 27% and specificity of 95% with NPV of 91% when using HbA1c cut-off value of 5.4% (36 mmol/mol).

Conclusions

Our results suggest that pregnant women with an HbA1c of $\geq 5.4\%$ (36 mmol/mol) should proceed with an OGTT. This may result in a significant reduction in the burden of testing on both patients and testing facility staff and resources. Further investigations are required to integrate and optimise the HbA1c as a single, non-fasting, screening tool for GDM.

Key words: HbA1c, glucose tolerance test, pregnancy, gestational diabetes, screening

The study was registered prospectively in the Australian New Zealand Clinical Trials Registry as a part of www.anzctr.org.au/ACTRN12611000739910.aspx trial.

Article summary

'Strengths and limitations of this study'

1. Oral glucose tolerance test (OGTT) is a standard screening test for gestational diabetes mellitus (GDM), however, it requires fasting overnight and 3 separate blood tests over 2 hours, is often poorly tolerated by pregnant women and is labour-intensive, adding an additional burden to an overstretched health system.
2. HbA1c is a simple, single, non-fasting test that may give insight to gestational diabetes.
3. Our study of 480 pregnant women suggests that HbA1c could be a useful screening tool for GDM.
4. HbA1c is safe, cost effective and more convenient for pregnant women. The diagnosis of GDM would be missed in few patients by using HbA1c as a screening tool.
5. The major effect on HbA1c is usually seen in the last 4-8 weeks of red cell age. Thus, it should be interpreted with caution if detecting a new diagnosis of GDM.

INTRODUCTION

Pregnancies affected by gestational diabetes mellitus (GDM) are at risk of developing a number of serious obstetric complications such as foetal growth abnormalities, shoulder dystocia, birth injury, prematurity and increased Caesarean section rate, as well as having long term implications for the wellbeing of mother and infant.¹ The risk of adverse perinatal and maternal outcomes is directly proportional to the degree of hyperglycaemia, with a linear relationship between maternal glucose and various neonatal outcomes.¹⁻²

The current screening process using the revised Australasian Diabetes in Pregnancy Society (ADIPS) guidelines published in 2013 based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria has resulted in an increase in the detected incidence of GDM in the Australian population from 6-8% to 13%.² The guidelines recommend a 75g oral glucose tolerance test (OGTT) at 24-28 weeks gestation for all pregnant women.

However, the OGTT is a cumbersome test that is time consuming, labour intensive and often poorly tolerated by pregnant women. The patient must be fasted, sit for over 2 hours and have at least 3 venepunctures. The gravida is prone to nausea and vomiting from delayed gastric emptying. This, coupled with gestational oedema compromising venous access, can lead to an invalid test result. Furthermore, the recommendation for universal screening has significantly increased the burden of testing.

The instability of blood glucose *ex vivo* leads to significant inter-laboratory variation of results. It is thought to vary by up to 14% in a third of cases.³

Whilst guidelines are in place, the glucose threshold values for diagnosis and methods of testing for GDM vary greatly from one institution to another. Moreover, as it is a specialised test, many collection centres do not provide this service, particularly in rural and remote locations, potentially disadvantaging an already vulnerable cohort of women.⁴

The need for a simpler, more universally acceptable and accessible test is becoming increasingly apparent. Glycosylated Hb, or HbA1c, is currently the most accepted measure of chronic glycaemia outside of pregnancy.

The National Health and Medical Research Council (NHMRC) guidelines 2009 recommend HbA1c to be the basis for diagnosis of Type 2 Diabetes Mellitus, with a value of 48 mmol/mol or 6.5% or greater being confirmatory.⁵

HbA1c is the product of an irreversible non-enzymatic binding of glucose to plasma proteins, specifically haemoglobin. The mean plasma glucose over the erythrocyte life span is correlated with a degree of glycosylation. It is a single, non-fasting blood test and reflects glucose levels over the previous 4-8 weeks. As compared to glucose testing it has been shown to have greater reliability with <6% inter-laboratory variation.³ Thus, HbA1c test has improved analytical stability with greater standardisation between assays and less pre-analytical variation. Further comparisons with fasting blood glucose and 2 hour post prandial glucose have shown HbA1c to have less intra-individual variation⁶ as it does not appear to be affected by diurnal variation, meals, fasting, acute stress or by the large number of common drugs known to influence glucose metabolism.⁷ The test is validated for a red cell survival time of approximately 3 months. Therefore, results need to be interpreted carefully in the clinical situation whereby erythrocyte half-life is significantly shortened by, for example, haemoglobinopathies, haemolysis, transfusion, anaemia and chronic renal failure.

Dilutional anaemia of pregnancy and increased erythrocyte turnover have, to date, hampered its acceptance as a tool for screening, if not diagnosis, of GDM.⁸

The accuracy of HbA1c as a screening test in pregnancy has been extensively studied over the last three decades and results have been inconsistent.⁹⁻¹³

Many of these studies were conducted prior to the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO 2008) findings,¹⁴ upon which the current International Association of Diabetes and Pregnancy Study Groups (IADPSG 2010)¹⁵ and ADIPS² screening strategies are based. Consequently, there is significant heterogeneity in both

methods used for screening for GDM and diagnostic glucose thresholds when compared with HbA1c. In addition, and importantly, many studies have used the same reference range for HbA1c in both pregnant and non-pregnant patients. Nonetheless, the results of these studies have been inconclusive. The overlap of HbA1c values between normal and GDM affected pregnancies has always been too great for HbA1c to have sufficient sensitivity and specificity to meet the screening requirements of a test.

Using current screening guidelines, Neilsen *et al* 2004 have shown the normal upper range of HbA1c in early pregnancy to be significantly lower than levels found outside of pregnancy, (5.7%/39 mmol/mol) and did not significantly differ from late pregnancy (5.6%/38 mmol/mol).¹⁶ This study looked at non-GDM women at 14 weeks and 33 weeks gestation as determined by negative OGTT values. Haemoglobin levels, however, were not accounted for and anaemia could have inadvertently lowered results.

O'Connor *et al* 2012 did find trimester specific reference intervals for HbA1c in a study of 246 non-diabetic pregnant women with normal haemoglobin levels: the first trimester range was 4.8-5.5% (29-37mmol/mol), second trimester 4.4-5.4% (25-36 mmol/mol), and third trimester 4.4-5.4% (25-36 mmol/mol).¹⁷

A meta-analysis of 43 studies, involving over 2812 GDM patients in China compared to 5918 controls concluded that, based on the Summary Receiver Operating Characteristic (SROC) curve analysis, HbA1c is a useful diagnostic tool for confirming GDM.¹⁸ Studies from 2001 to 2012 were included in this meta-analysis with varying diagnostic criteria for GDM and cut off values for HbA1c, such that the authors recommended HbA1c to be tested in parallel with conventional tests.¹⁸

Our current study is focusing on parallel prospective comparison between the HbA1c and standard OGTT when used as a screening tool in pregnancy for GDM.

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Objectives of the study:

The aim of the study was to provide an objective assessment of the utility of HbA1c when used as a screening tool in pregnancy. A direct comparison of HbA1c levels with results of the OGTT in women, tested concurrently at the 24-28 gestational week, was undertaken.

PATIENTS AND METHODS

Participants

We recruited 480 pregnant women during the period from September 2012 to July 2014. For these patients, we performed a simultaneous prospective assessment of HbA1c versus standard OGTT in a cohort of consecutive pregnant women presenting to the Launceston General Hospital (a tertiary referral teaching hospital in Tasmania, Australia). Pregnant women were approached when attending their routine third trimester OGTT test at our institution. Written informed consent was obtained from all subjects. For these patients, simultaneous Full Blood Count (FBC) and iron studies were performed as per our routine antenatal assessment. Median and mean age of participants was identical 29 years (range, 18-47).

The trial was approved by the Tasmanian Human Research Ethics Committee as a part of another trial targeting the same population studying thyroid disease of pregnancy and was registered in the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au/ACTRN12611000739910.aspx) and the World Health Organization Clinical Trials Registry (www.who.int/trialsearch/Trial2.aspx?trialid=ACTRN12611000739910).

Inclusion criteria

All sequential pregnant women who were ≥18 year old and presented for OGTT test at 24-28 weeks gestation in our tertiary referral hospital were offered the trial. The OGTT was performed according to our policy at the LGH at the time of study.

Exclusion Criteria

Twin pregnancies were excluded as well as women with an early diagnosis of GDM i.e. prior to 24 weeks gestation as this may create unknown bias to the results of the trial.

Parameters such as gravity, parity, ethnicity, personal and family history of diabetes, type of delivery, complications of pregnancy, body mass index and perinatal data, as well as data regarding the infants' APGAR scores, weight and sex were collected prospectively from women enrolled in the trial after informed consent.

OGTT was performed according to our standard protocol. The patient was required to have been in good health and to be consuming a normal diet, particularly with regard to carbohydrate intake (>150g/day). The test was performed after an overnight fast of 10 hours. The test was commenced before 10am and the patient remained resting quietly for the duration of the OGTT. Blood samples were collected into Becton Dickinson 2mL Fluoride Oxalate Vacutainer tubes. A sample was collected at baseline and then the patient consumed the 75g glucose load. We used a commercially available product containing 75g of dextrose in 300mL carbonated liquid (SteriHealth Gluco Scan 75g). The patient was required to consume the whole volume within five minutes of commencing the drink. Further blood samples were collected at one hour and two hours post commencement of the dextrose drink. Glucose was measured within three hours of collection of the sample using the Abbott glucose hexokinase method on an Architect C8000 analyser (Abbott Australasia Pty. Ltd.).

HbA1c samples were collected into Becton Dickinson 4mL K2EDTA Vacutainer tubes.

HbA1c was measured by immunoassay using the DCA 2000 (Siemens Ltd., Marburg, Germany). The DCA 2000 analyser measures HbA1c standardised to the National Glycohemoglobin Standardization Program (NGSP), which is in turn aligned to the Diabetes Control and Complications Trial (DCCT) results with international standardisation as set by the International Federation of Clinical Chemistry (IFCC) (<http://www.ngsp.org/certified.asp>).

GDM was defined as present if fasting blood glucose was ≥ 5.1 mmol/L or GTT 1hr ≥ 10.0 mmol/L OR GTT 2 hr ≥ 8.5 mmol/L. GDM was defined as not present if fasting blood glucose was < 5.1 mmol/L and GTT 1hr < 10 mmol/L and GTT 2 hr < 8.5 mmol/L. The diagnostic criteria are defined by the 2013 Australasian Diabetes in Pregnancy Society (ADIPS) consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia.^{5,19}

STATISTICAL ANALYSIS

Patient characteristics were expressed as percentages for categorical variables, and as mean and standard deviation, or median with interquartile range for continuous variables.

Groups were compared using *t*-test, and multiple-category variables were analysed using analysis of variance.

Bland-Altman plot was used to assess the agreement between HbA1c levels and the values of the glucose tolerance test. Receiver operating characteristic (ROC) curve was performed to assess the discriminative capacity of HbA1c for detection of GDM. Sensitivity, specificity, predictive values, false positive, and negative rates were calculated. All analyses were performed with SAS (version 9.3).

RESULTS

We recruited 480 women with a median 26 and mean of 25.7 weeks gestation. Approximately 12% of our cohort were diagnosed with GDM according to the ADIPS criteria and this is consistent with the expected incidence of GDM in Australia. The median gestational age at enrolment was 26 weeks with a mean of 25.7 weeks (SD 3.3) with a mean fasting glucose level of 4.37 mmol/L (SD 0.46), 6.85 mmol/L (SD 1.7) at 1 hour and 5.84 mmol/L (SD 1.45) at 2 hours. The mean HbA1c was 4.8 % (29mmol/mol) (SD 0.36). FBC and iron studies were assessed in the same subjects, showing median Hb of 119 g/L (range, 92-145) and median ferritin of 12 µg/L (range, 1-204).

Spearman correlation between OGTT and HbA1c showed significant association of 1h and 2h OGTT with HbA1c ($p < 0.0001$) (Table 1). However, no such correlation existed for Hb and OGTT ($p = 0.38$ and 0.25 respectively). Further analyses demonstrated that ferritin and Hb levels correlated with HbA1c ($p = 0.02$, $p < 0.0001$ respectively) (Table 1). Bland-Altman correlations between OGTT and HbA1c are shown in Figures 1 and 2.

Table 1: Spearman correlations between OGTT and HbA1c

	Spearman Correlation Coefficients					
	Prob > r under H0: Rho=0					
	Number of Observations					
	Fasting Blood Glucose	OGTT (mmol/L) 1 HR	OGTT (mmol/L) 2 HR	HbA1c %	Ferritin	Hb
Fasting blood glucose	1.00000					
OGTT (mmol/L)/1Hr	0.36109 <.0001 475	1.00000				
OGTT (mmol/L) 2 Hr	0.31745 <.0001 480	0.61037 <.0001 475	1.00000			
HbA1c %	0.34298 <.0001 438	0.21911 <.0001 434	0.22906 <.0001 438	1.00000		
Ferritin	0.03316 0.4757 465	0.11589 0.0129 460	0.00636 0.8912 465	-0.10974 0.0233 427	1.00000	
Hb	-0.03201 0.4860 476	0.03996 0.3869 471	-0.05220 0.2557 476	-0.26569 <.0001 436	0.23075 <.0001 463	1.00000

The number of women in our cohort who had GDM according to the OGTT criteria was 57, representing 11.9% of the 480 studied pregnant women. Overall, in the same trial period at our laboratory, there were 97 out of 1795 pregnant women (5.4%) tested for OGTT who had a fasting blood glucose level > 5.1 mmol/L while at 2 h the glucose level was > 8.5 mmol/L in 96 out of 1775 patients (5%).

It is worth noting that most of the patients studied (88%) were iron deficient with ferritin level $<30 \mu\text{g/L}$, while 57% had ferritin $<15 \mu\text{g/L}$ similar to the results of previous trials.²⁰⁻²² The HbA1c distribution by GDM status is shown in Figure 3.

Using an arbitrary cut-off value for HbA1c at 5.1% (32 mmol/mol) to detect GDM showed sensitivity of 61% and specificity of 68% with negative predictive value (NPV) of 93% versus sensitivity of 27% and specificity of 95% with NPV of 91% when using a HbA1c cut-off value of 5.4% (36 mmol/mol) (Table 2).

Table 2: Sensitivity and Specificity for all values of HbA1c

HbA1c %	Sensitivity	Specificity	PPV	NPV
10	0	0.997	0	0.888
6.1	0.02	0.997	0.5	0.89
6	0.041	0.997	0.667	0.892
5.9	0.061	0.997	0.75	0.894
5.8	0.082	0.997	0.8	0.896
5.7	0.102	0.995	0.714	0.898
5.6	0.122	0.99	0.6	0.9
5.5	0.224	0.982	0.611	0.91
5.4	0.265	0.954	0.419	0.912
5.3	0.347	0.884	0.274	0.915
5.2	0.551	0.797	0.255	0.934
5.1	0.612	0.676	0.192	0.933
5	0.694	0.519	0.154	0.931
4.9	0.735	0.314	0.119	0.904
4.8	0.816	0.18	0.111	0.886
4.7	0.959	0.1	0.118	0.951
4.6	0.959	0.046	0.112	0.9

PPV=positive predictive value; NPV=negative predictive value

Regarding ethnicity, 93% of the population studied were Caucasian, with 4% Asian and 3% Aboriginal. There was no significant correlation between GDM or HbA1c and ethnicity. Nevertheless, this study is not statistically powered to detect such differences as other trials demonstrated.²³

The sex of the baby, complications of pregnancy or type of delivery did not show an association to HbA1c levels. However, GDM itself was associated with more

complications during pregnancy ($p < 0.0001$) compared to non-GDM pregnant women but did not influence intra or postpartum or natal complications (Table 3).

Table 3: Does GDM status influence complications during pregnancy, intrapartum postpartum?

		GDM status (N, %)		P value*
		0= No gestational diabetes	1= Gestational diabetes	
Complications During Pregnancy	1 = No	305 (97)	8 (3)	<.0001
	2 = Yes	95 (68)	45 (32)	-
	3 = Insufficient information	21 (91)	2 (9)	-
Intrapartum complications	1 = No	378 (88)	52 (12)	0.8203
	2 = Yes	20 (87)	3 (13)	-
	3 = Insufficient information	23 (92)	2 (8)	-
Postpartum complications;	1 = No	362 (88)	50 (12)	0.9047
	2 = Yes	36 (88)	5 (12)	-
	3 = Insufficient information	23 (92)	2 (8)	-

*Groups were compared with Chi-Square or Fisher tests.

Proposed cost saving

The use of HbA1c in the context of gestational diabetes screening provides an economy at the point of specimen collection. The resource requirement for the specimen collection facility may be significantly reduced, for example; bleeding a patient once for an HbA1c as opposed to three times for a GTT (Baseline, 1 hour and 2 hour). Consumables and equipment, salaries and accommodation are also reduced which may, over time, support an economic argument.

Cost of OGTT and HbA1c

Although a cost analysis was not the primary objective of the study, it is worthwhile attempting to calculate approximate costs of Consumables/Equipment/Accommodation per bleed.

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In our laboratory, the cost of reagents and consumables to perform an HbA1c is approximately USD \$9.00. This is compared to the cost of performing the three glucose tests as part of an OGTT which is in the order of \$0.50.

This increased cost of laboratory consumables is more than offset by the reduction in labour and infrastructure costs of collecting a single sample for HbA1c compared to performing the OGTT.

At the LGH public laboratory, the laboratory consumable cost per bleed is approximately \$2 USD and the salary of the nurse performing the venepuncture is approximately \$10 per bleed. The cost of testing an additional blood sample is approximately \$9 per given episode. It therefore costs \$12.00 to perform the single blood collection for an HbA1c and \$34 for the OGTT. In addition, the 75g glucose load for the OGTT costs \$2. The overall cost of performing the two procedures is approximately \$21 for an HbA1c and \$36.50 for an OGTT, a saving of \$15.50 per episode for HbA1c compared to OGTT.

Furthermore, in health systems that often have resourcing issues such as staff and accommodation, it is far more efficient to have a patient bled once rather than three times and accommodate them (and their party) in the waiting room for more than two hours.

Furthermore, there is an economy for the patients themselves in terms of time off work, parking and issues such as child care when applicable.



DISCUSSION

Since the time of the original publications investigating the value of HbA1c for GDM diagnosis, laboratory testing of HbA1c has become increasingly standardised and evolved to be a simpler, more accurate and automated test. It has developed from simple ELISA to turbidometric inhibition immunoassay (TINIA), an assay method largely unaffected by either haemoglobinopathies or uraemia, through to precision liquid chromatography. Previously, HbA1c results could not be compared between one laboratory and another let alone one country to another.²⁴ However, since international standardisation of the assay, this is no longer the case.

Rajput *et al* 2012 studied 607 women between 24-28 weeks' gestation, similar to our study. They were evaluated for GDM using OGTT based on ADA criteria (2hr 75g OGTT or "one step strategy") and concurrently tested for HbA1c.²⁵ A cut-off value of $\geq 5.4\%$ (36 mmol/mol) had a sensitivity of 85.7% and specificity of 61.1%. Only 2.8% would have been diagnosed incorrectly as GDM and reporters state that it would have obviated the need for OGTT in 61.8% of cases.²⁵ In a smaller retrospective study of 145 high risk Saudi Arabian women, Aldasouqi *et al* in 2008 demonstrated the use of HbA1c in detecting 87% of the GDM patients diagnosed on OGTT, missing 12%. However, in this study the cutoff HbA1c was 6% (42 mmol/mol).²⁶

Other trials determined that the normal mean HbA1c values in Asian Indian women ranged between 5.36% \pm 0.36% and $\geq 6\%$ (42 mmol/mol) in women with GDM in a study of 507 women.²⁷ This study was interesting in that all trimesters were studied. Women who had a positive HbA1c but negative OGTT in the first trimester subsequently developed GDM.²⁷ The implication of this is twofold. First, the HbA1c false positives identified in other studies may have actually been true positives with OGTT results being false negatives. Second, if the results of this study are reproducible, then management instituted earlier may have an impact on outcome for babies of mothers who, despite being screened, detected and treated during third trimester, still suffer the consequences of GDM.

Genetically determined variations in the degree of glycosylation of haemoglobin, independent of glycaemia, are thought to exist and are reflected in ethnic differences in HbA1c levels.⁸ This suggests that population reference ranges need to be

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established prior to universally implementing HbA1c as a screening test in gestational diabetes. It is worth noting that the vast majority of our studied population were Caucasian (94%).

The use of HbA1c as a screening tool for gestational diabetes has yet to be evaluated in an Australian population. To our knowledge, there are no published data comparing the 75g 2 step OGTT as per current guidelines with HbA1c in establishing the diagnosis of GDM. It is anticipated that validating the diagnostic utility of HbA1c in pregnancy in Australia would result in a reduction in the burden of testing, increase patient access and compliance, therefore facilitate the management of the gestational diabetic mother and perhaps improve perinatal and maternal outcomes.

We found that the application of HbA1c as a method for screening GDM within the Australian population needs further refining and definition for its use. Outside of pregnancy, it has been established that HbA1c measurement can accelerate and facilitate patient screening, diagnosis and management of diabetes. The usual cut-off value for diagnosing diabetes outside pregnancy seems to be much higher than the cut-off value needed to diagnose diabetes associated with pregnancy. This is in concordance with other studies already detailed.

The results of our study indicate that using an HbA1c level of 5.4% (36 mmol/mol) at third trimester (26 week) has a specificity of 95%, sensitivity of 27%, and NPV of 91% in detecting GDM, in line with the reference range for pregnancy reported by other studies.^{17,25} Using a cut-off value of HbA1c 5.1% (32 mmol/mol), the sensitivity increased to 55%, at the expense of specificity, which decreased to 80%. The positive association between HbA1c and OGTT is an advantage, but the low sensitivity of HbA1c becomes a hurdle in standardising such a test in pregnancy.

Further investigations are required to integrate HbA1c as a single, non-fasting diagnostic test for GDM. However, the high NPV may make it useful as an initial screening test. For example, patients with an HbA1c >5.4% (36 mmol/mol) should proceed with an OGTT. This alone would generate a significant reduction in the burden of testing.

It is worth noting that the additional disadvantages of OGTT were potentially avoided by the use of HbA1c. These include the need to fast, non-tolerance of glucose ingestion, nausea, a more than 2 hour stay in the laboratory, multiple venipunctures, associated stress and discomfort, greater use of consumables and finally, increased time requirements on the blood nurse and the associated costs.

Our study has a few shortcomings due to the lack of sensitivity of HbA1c and that some patients, although a small number, will be missed in diagnosis of GDM. Therefore, further optimisation of the test is required prior to its application as a screening tool for GDM. It is likely that the pathophysiology of GDM is different from DM in the general population. However, GDM may be an indicator of increased risk of non-insulin dependent type 2 DM in the postnatal period. Pregnancy is a state of insulin resistance. Hormones secreted by the placenta, including growth hormone, corticotropin releasing hormone, placental lactogen and progesterone, all act to increase insulin resistance in the mother, serving to ensure adequate supply of nutrients to the developing foetus.²⁸ Where the mother has insufficient pancreatic function to cope with this increasing insulin resistance, diabetes ensues.

In contrast, in Type 2 (non-pregnant) diabetes mellitus, increasing insulin resistance obviously is not mediated by a placenta but rather by a complex interplay between genetic predisposition, obesity and decreased physical activity. Abdominal fat is metabolically active, producing hormones that promote insulin resistance. Leptin, tumor necrosis factor, alpha and resistin are among the many “adipokines” implicated in insulin resistance and subsequent development of Type 2 DM.²⁹⁻³¹ Adipose cells, furthermore, are thought to trigger chronic inflammation, which in turn contributes to the development of insulin resistance.³²

However, the difference in the performance of HbA1c in non-pregnant versus pregnant populations may not be entirely explained by different pathophysiology.

In summary, employing a cut-off value for HbA1c at 5.4 % (36 mmol/mol) for detecting GDM showed NPV of 91% and specificity of 95%. Similar results could be achieved with HbA1c level >5.1% as a screening tool for GDM. The high specificity

and NPV value may be useful as an initial screening test for GDM. This may result in significant reduction in the burden of testing on both patients and testing facility, staff and resources. Further investigations are required to integrate HbA1c as a single non-fasting screening tool for GDM with optimisation of the cut-off value.

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study design; collection, analysis, and interpretation of data; writing the research report; and

the decision to submit the manuscript for publication. This is an investigator-initiated study.

All aspects of the study conception, design, administration, data collection, analysis

and presentation has been undertaken independently of any sponsor.

Contributorship Statement

Alhossain A. Khalafallah is the principal investigator of the study who organised and coordinated all aspects of the research including all steps of the manuscript preparation. He is responsible for the study concept, design, writing, reviewing, editing and approving the manuscript in its final form.

Eileen Phuah, Abdul Majeed Al-Barazan, Irena Nikakis and Andrea Radford recruited the patients and contributed in the study design, analysis and interpretation of data, writing the manuscript and reviewed and approved the manuscript in its final form.

Wade Clarkson, Clinton Trevett, Terry Brain, Val Gebiski and Anne Corbould conducted the testing supervised the patients at the Pathology Dept., Launceston General Hospital and drafted and finally approved the manuscript.

Data Sharing Statement: No additional data available

REFERENCES

1. Casey Bm, Lucas MJ, McIntire DD et al. Pregnancy outcomes in women with gestational diabetes compared with the general obstetric population. *Obstet Gynecol* 90:869-873

2. Nankervis A, McIntyre H, Moses R, Ross G, Callaway L, Porter C, et al. Australasian Diabetes in Pregnancy Society (ADIPS) consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia. 2012.

3. d'Emden M. Glycated haemoglobin for the diagnosis of diabetes. *Australian Prescriber*. 2014;37:98-100

4. Hoang H, Le Q. Comprehensive picture of rural women's needs in maternity care in Tasmania, Australia. *Aust J Rural Health*. 2013;21:197-202

5. Colaguri S D, D, Girgis S et al. National evidence based guideline for case detection and diagnosis of type 2 diabetes. Diabetes Australia and National Health and Medical Research Council; Canberra 2009

6. Selvin E, Crainiceanu CM, Brancati FL, Coresh J. Short-term variability in measures of glycemia and implications for the classification of diabetes. *Arch Intern Med*. 2007;167:1545-51

7. Pandit MK, Burke J, Gustafson AB, Minocha A, Peiris AN. Drug-induced disorders of glucose tolerance. *Ann Intern Med*. 1993;118:529-39

8. Lippi G, Targher G. Glycated hemoglobin (HbA1c): old dogmas, a new perspective? *Clin Chem Lab Med*. 2010;48:609-14

9. Agarwal MM, Hughes PF, Punnose J, Ezimokhai M, Thomas L. Gestational diabetes screening of a multiethnic, high-risk population using glycated proteins. *Diabetes Res Clin Pract*. 2001;51:67-73

10. Agarwal MM, Dhath GS, Punnose J, Koster G. Gestational diabetes: a reappraisal of HBA1c as a screening test. *Acta Obstet Gynecol Scand*. 2005;84:1159-63

11. Pollak A, Brehm R, Havelec L, Lubec G, Malamitsi-Puchner A, Simbrunner G, et al. Total glycosylated hemoglobin in mothers of large-for-gestational-age infants: a postpartum test for undetected maternal diabetes? *Biol Neonate*. 1981;40:129-35

12. Griffiths RJ, Vinall PS, Stickland MH, Wales JK. Haemoglobin A1c levels in normal and diabetic pregnancies. *Eur J Obstet Gynecol Reprod Biol*. 1987;24:195-200

13. Moses RG. HbA1c and the diagnosis of gestational diabetes mellitus--a test whose time has not yet come. *Diabetes Res Clin Pract*. 2012;98:3-4

14. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;358:1991-2002

15. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, 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
16. Nielsen LR, Ekbom P, Damm P, Glumer C, Frandsen MM, Jensen DM, et al. HbA1c levels are significantly lower in early and late pregnancy. *Diabetes Care*. 2004;27:1200-1
17. O'Connor C, O'Shea PM, Owens LA, Carmody L, Avalos G, Nestor L, et al. Trimester-specific reference intervals for haemoglobin A1c (HbA1c) in pregnancy. *Clin Chem Lab Med*. 2012;50:905-9
18. Tian QW, Xuan C, Wang HW, Zhao JX, Yu WL, Gao G, et al. Diagnostic accuracy of glycosylated hemoglobin in chinese patients with gestational diabetes mellitus: a meta-analysis based on 2,812 patients and 5,918 controls. *Genet Test Mol Biomarkers*. 2013;17:687-95
19. <http://adips.org/downloads/ADIPSConsensusGuidelinesGDMACCEPTEDFINAL.pdf>
20. Khalafallah AA, Dennis A, Bates J, Bates G, Robertson IK, Smith L, et al. Three-year follow-up of a randomised clinical trial of intravenous versus oral iron for anaemia in pregnancy. *BMJ Open*. 2012 Oct 18;2(5). pii: e000998. doi: 10.1136/bmjopen-2012-000998. Print 2012. PMID: 23087011
21. Khalafallah AA, Dennis AE. Iron deficiency anaemia in pregnancy and postpartum: pathophysiology and effect of oral versus intravenous iron therapy. *J Pregnancy*. 2012;2012:630519. doi: 10.1155/2012/630519. Epub 2012 Jun 26. Review. PMID: 22792466
22. Khalafallah AA, Dennis AE, Ogden K, Robertson I, Charlton RH, Bellette JM, et al. A prospective randomized, controlled trial of intravenous versus oral iron for moderate iron deficiency anaemia of pregnancy. *J Intern Med*. 2010 Sep;268(3):286-95. doi: 10.1111/j.1365-2796.2010.02251.x. Epub 2010 May 19. PMID: 20546462
23. Hartland AJ1, Smith JM, Clark PM, Webber J, Chowdhury T, Dunne F. Establishing trimester- and ethnic group-related reference ranges for fructosamine and HbA1c in non-diabetic pregnant women. *Ann Clin Biochem*. 1999; 36:235-7.
24. Gillery P. A history of HbA1c through Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2013;51:65-74
25. Rajput R, Yogesh Y, Rajput M, Nanda S. Utility of HbA1c for diagnosis of gestational diabetes mellitus. *Diabetes Res Clin Pract*. 2012;98:104-7
26. Aldasouqi SA, Solomon DJ, Bokhari SA, Khan PM, Muneera S, Gossain VV. Glycohemoglobin A1c: A promising screening tool in gestational diabetes mellitus. *Int J Diabetes Dev Ctries*. 2008;28:121-4
27. Balaji V, Madhuri BS, Ashalatha S, Sheela S, Suresh S, Seshiah V. A1C in gestational diabetes mellitus in Asian Indian women. *Diabetes Care*. 2007;30:1865-7

28. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr.* 2000;71:1256S-61S

29. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA.* 2009;302:179-88

30. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science.* 1993;259:87-91

31. Stepan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature.* 2001;409:307-12

32. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med.* 2011;17:179-88

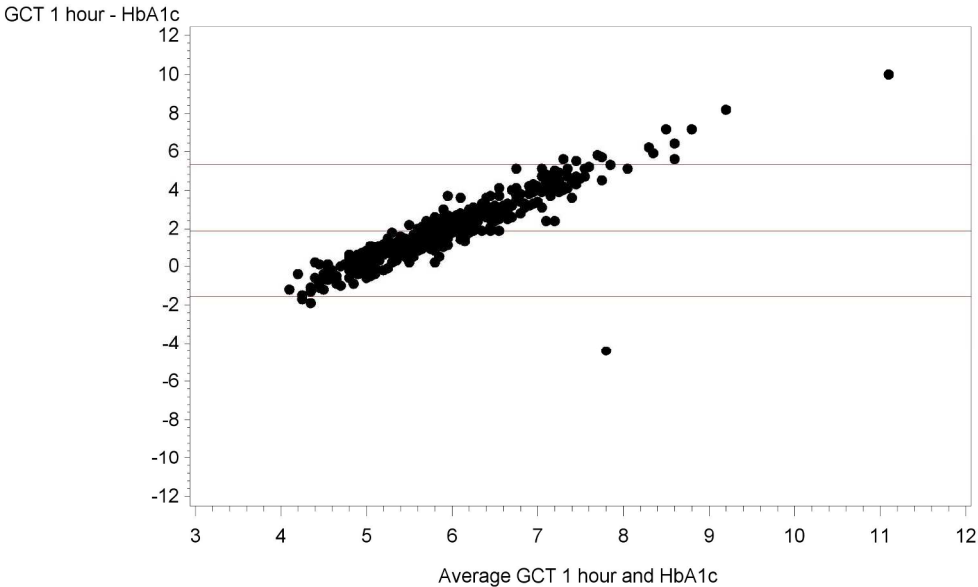
Figure 1. Legend
Bland-Altman plot, OGTT 1 hour vs HbA1c

Figure 2. Legend
Bland-Altman plot, OGTT 2 hour vs HbA1c

Figure 3. Legend
HbA1c distribution by GDM status

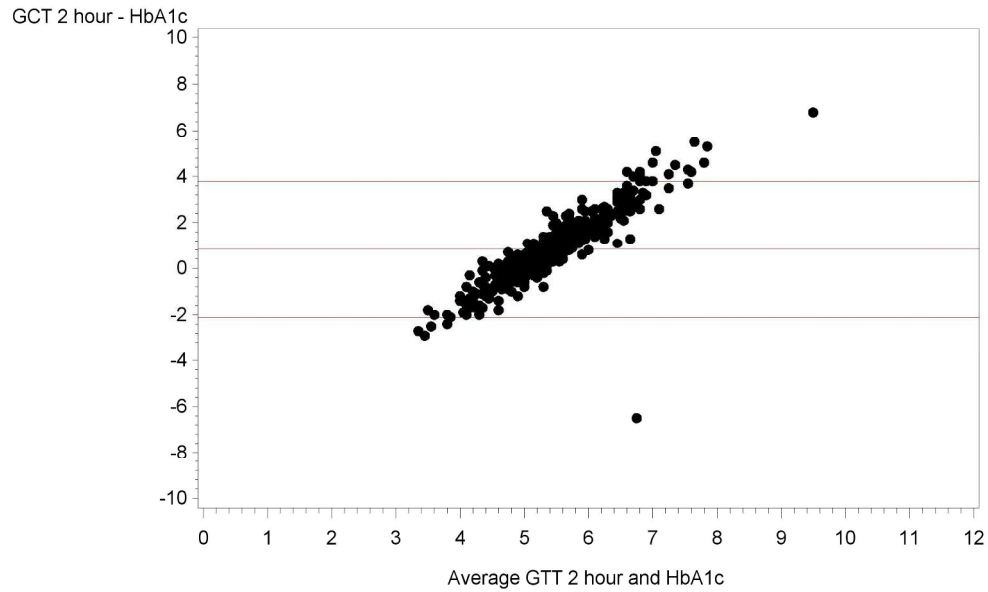
For peer review only

Bland-Altman Plot

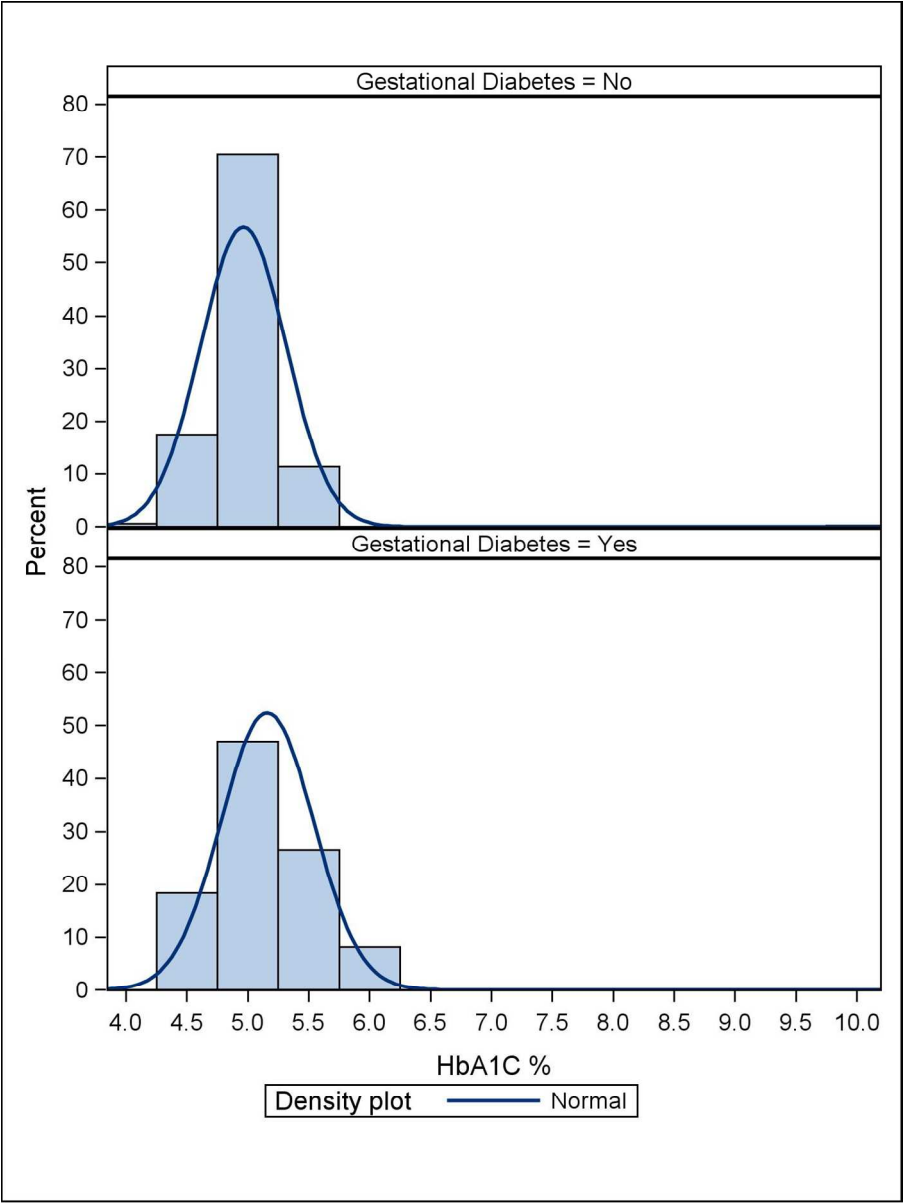


Bland-Altman plot, OGTT 1 hour vs HbA1c
211x158mm (300 x 300 DPI)

Bland-Altman Plot



Bland-Altman plot, OGTT 2 hour vs HbA1c
211x158mm (300 x 300 DPI)



HbA1c distribution by GDM status
127x169mm (300 x 300 DPI)

Section & Topic	No	Item
TITLE OR ABSTRACT		Glycosylated Haemoglobin A1c for screening and diagnosis of gestational diabetes
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy sensitivity, specificity, predictive values
ABSTRACT		
	2	Structured summary of study design, methods, results, and conclusions; page 2
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test; page 5
	4	Study objectives and hypotheses; page 6
METHODS		
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<i>Participants</i>	6	Eligibility criteria; page 7
	7	On what basis potentially eligible participants were identified; page 7 (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates); page 7
	9	Whether participants formed a consecutive, random or convenience series; page 7
<i>Test methods</i>	10a	Index test, in sufficient detail to allow replication; page 4
	10b	Reference standard, in sufficient detail to allow replication; page 4
	11	Rationale for choosing the reference standard (if alternatives exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory; page 5
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory; not applicable
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test; page 8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard; page 8
<i>Analysis</i>	14	Methods for estimating or comparing measures of diagnostic accuracy; page 9
	15	How indeterminate index test or reference standard results were handled; page 10
	16	How missing data on the index test and reference standard were handled; not applicable
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory; pages 9-11
	18	Intended sample size and how it was determined; observational study only
RESULTS		
<i>Participants</i>	19	Flow of participants, using a diagram; not applicable
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	21b	Distribution of alternative diagnoses in those without the target condition; page 13
	22	Time interval and any clinical interventions between index test and reference standard; page 14
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	25	Any adverse events from performing the index test or the reference standard; pages 14-15
DISCUSSION		
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalizability; page 15
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OTHER INFORMATION		
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