

Role of HbA1c in diagnosis of gestational diabetes mellitus

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Abstract

Objective: To evaluate glycated haemoglobin as a biomarker for diagnosing gestational diabetes mellitus while keeping the oral glucose tolerance test as the gold standard.

Methods: The cross-sectional study was conducted from January, 2016, to January, 2018, at PNS Hafeez Hospital, Islamabad, Pakistan and comprised of pregnant subjects who were first subjected to 2-hour oral glucose tolerance test along with the first evaluation of glycated haemoglobin. Clinical evaluation, including history and measurements of anthropometric indices and blood pressure, were also done. On the basis of the results, the subjects were grouped as those having gestational diabetes mellitus (group A) and those without it (group B). Data was analysed using SPSS 15.

Results: Of the 280 subjects, gestational diabetes mellitus was found in 50(17.85%). Differences in glycated haemoglobin between the groups was significant ($p < 0.002$). Glycated haemoglobin test provided sensitivity of 70% and specificity of 84.78%.

Conclusion: With due adjustments, glycated haemoglobin testing can help in reducing the frequency of oral glucose tolerance test.

Keywords: HbA1c, Gestational diabetes mellitus, 2-hour OGTT. (JPMA 70: 1731; 2020) DOI: <http://doi.org/10.5455/JPMA.24080>

Introduction

Gestational diabetes mellitus (GDM) is a condition diagnosed for the first time during pregnancy.¹ It has been established that gestational hyperglycaemia is associated with multiple adverse outcomes for both obstetric and neonatal consequences. The non-desirable outcomes for the child include intra-uterine death (IUD), macrosmia, hypoglycaemia and hypocalcaemia, while maternal complications of GDM include increased hypertensive episodes, operative deliveries and incidence of type-2 diabetes mellitus (T2DM) in future.² Moreover, the offsprings of GDM mothers are prone to develop metabolic diseases in the later phase of life, like Diabetes Mellitus (DM) and obesity.² Timeliness and accuracy in diagnosis, therefore, remain pivotal and major drivers in the effective intervention and management of GDM.

The current concept of GDM diagnosis relies solely upon establishing hyperglycaemia in the mother. Over the preceding decade, scientific evidence has introduced varying modalities to screen and diagnose GDM. From O' Sullivan's 50gm glucose challenge test (GCT) to the National Diabetes Data Group (NDDG) criteria for diagnosing GDM in 1979, the Carpenter and Coustan criteria, and the latest is the International Association of Diabetes in Pregnancy

Study Group (IADPSG) methodology.³⁻⁶ Still, the diagnostic criteria for GDM suffers from lack of consensus in terms of several cut-offs with varying regional recommendations.⁷ These tests primarily rely upon plasma glucose readings at fasting or at various intervals after glucose loading.⁴⁻⁶ Over the last 4 decades, this conventional evidence has mostly prevailed and has demonstrated reasonable association and correlation with foeto-maternal risks in pregnancy.^{2,8,9} Provided the diagnostic performances acceptable in many ways, these glucose loading tests have inherent limitations like prolonged waiting time, multiple sampling and issues pertaining to glucose load tolerance in pregnant women.¹⁰ Secondly, the recommendations simply suggest uniform glucose (50, 75 or 100 grams) loading to all pregnant subjects regardless of body-weight, age and patient's other confounding factors, like familial predisposition towards DM which can result in false positive (FP) or false negative (FN) results not helping the targeted outcome.¹¹ Furthermore, biotechnology and internal level external quality assurance programmes, like the National Glycohaemoglobin Standardisation Programme (NGSP) have improved the diagnostic performance of biomarkers like glycated haemoglobin (HbA1c) which can help predict possible diabetogenic tendencies in GDM.¹² Moreover, amidst all current controversial data on HbA1c performance as a diagnostic tool in pregnancy, studies have highlighted the utility of HbA1c in the diagnosis of GDM.^{13,14} Regional data on the subject is limited, but does indicate the variation due to race, age and region. However, Khan R et al. though not a primary endpoint, have highlighted very high HbA1c

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values among GDM patients in comparison to healthy pregnant women.¹⁵ Similarly, Shobha P et al.¹⁶ have also shown association between HbA1c results and unfavourable pregnancy outcomes. Bhavadharini B et al. have also shown raised HbA1c levels in females delivering babies with macrosmia.¹⁷

The current study was planned to evaluate the role of HbA1c in the screening of GDM while keeping The International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria of 2-hour oral glucose tolerance test (OGTT) results as the primary endpoint.

Subjects and Methods

The cross-sectional study was conducted from January, 2016, to January, 2018, at PNS Hafeez Hospital, Islamabad, Pakistan and was part of a larger cross-sectional analysis carried out by the Departments of Gynaecology and Obstetrics and Pathology at the same institution from July, 2016, to January, 2018. After approval from the institutional board, pregnant subjects who visited the Department of Gynaecology and Obstetrics were screened during booking and later at regular intervals and subjects were requested to volunteer for the study during mid-pregnancy. Subjects who volunteered and reported in exact medical fasting status to the Department of Pathology for evaluation were enrolled for further testing after written informed consent was obtained from each of them. Subjects who finally consented were evaluated for suitability by taking history. Patients having pregnancy duration <16 weeks or >32 weeks, known diabetes mellitus (DM) hypertension (HTN) or other chronic disorder or some acute ailments were excluded. Over the next 18 months, the subjects were finally tested for 2-hour OGTT with 75 grams glucose load with sampling carried out in fasting, 1-hour and 2-hour after glucose load as per the IADPSG criteria.⁶ Fasting specimens were accompanied by HbA1c test. At this stage, some subjects were again excluded on account of multiple reasons, including vomiting during tests, showing inability to complete 2-hour follow-up after glucose load, sampling issue during testing and not presenting back for re-testing. Plasma glucose samples were collected in fluoride bottles and HbA1c samples were collected in ethylenediamine tetraacetic acid (EDTA) containers. Glucose was measured on Selectra-ProM by glycerol-3-phosphate oxidase- phenol + aminophenazone (GPO-PAP) method and HbA1c was measured by chemiluminescent micro particle immunoassay (CMIA) on ARCHITECT iSystem (Abbot Diagnostics). Blood complete picture (CP) was also done from EDTA samples to exclude subjects with anaemia.

Subjects were classified into 2 groups as either having GDM group A or not having GDM group B as per the recommended cut-offs of the IADPSG criteria.⁶

The entire dataset was entered into Microsoft Excel programme and later transferred into SPSS 15. Descriptive statistics in terms of frequency and percentages as well as mean + standard deviation (SD) were calculated for age, parity status, number of children, previous operative delivery (Caesarean section) and abortions. Differences between subjects with GDM and without GDM were evaluated through independent sample t-test. Pearson correlation was utilised to see correlation between various demographic, anthropometric and biochemical parameters. Receiver operating characteristic (ROC) curve analysis was performed for HbA1c with results of IADPSG criteria using 2-hour OGTT with 75 grams glucose load as the gold standard. Mean HbA1c values for subjects with and without GDM from the independent sample t-tests i.e., 5.36% and 6.06% were kept as cut-offs for HbA1c to evaluate diagnostic performance against the 2-hour OGTT diagnosis. ROC curve analysis was utilised to see the area under the curve (AUC) for HbA1c against IADPSG-defined 2-hour OGTT.⁶ The effect of BMI, being significantly different between subjects with and without GDM, was evaluated through general linear model (GLM) analysis with GDM diagnosis as independent factor for HbA1c results. Finally, regression analysis was carried out to see the contribution of BMI and age along with GDM diagnosis for HbA1c build-up.

Results

Of the 300 subjects evaluated, 280(93%) completed the

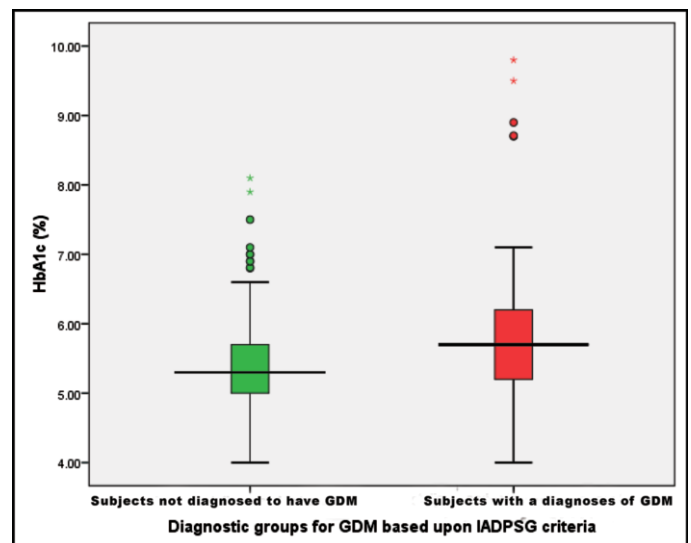


Figure-1: Differences in glycated haemoglobin (HbA1c) results between subjects diagnosed to have gestational diabetes mellitus (GDM) [Mean= 6.06, SD=1.495, (n=50)] and not diagnosed to have GDM [Mean= 5.36, SD=0.625, (n=230)], $p<0.002$.

Table-1: Correlation between glycated haemoglobin (HbA1c) and demographic, anthropometric and biochemical parameters.

Parameter	HbA1c (%)	
Age (Years)	Pearson Correlation	0.144*
	Sig. (2-tailed)	0.016
	N	279
Body mass Index (BMI)	Pearson Correlation	0.130*
	Sig. (2-tailed)	0.031
	N	275
Systolic blood pressure (mm of Hg)	Pearson Correlation	-0.056
	Sig. (2-tailed)	0.357
	N	272
Diastolic blood pressure (mm of Hg)	Pearson Correlation	-0.066
	Sig. (2-tailed)	0.278
	N	272
Fasting plasma glucose (mg/dl)	Pearson Correlation	.490**
	Sig. (2-tailed)	<0.001
	N	280
Plasma glucose result 1-hour after 75-gram glucose load (mg/dl)	Pearson Correlation	.455**
	Sig. (2-tailed)	<0.001
	N	280
Plasma glucose result 2-hour after 75-gram glucose load (mg/dl)	Pearson Correlation	.464**
	Sig. (2-tailed)	<0.001
	N	280
Serum Alanine transaminase (ALT) (IU/L)	Pearson Correlation	0.025
	Sig. (2-tailed)	0.676
	N	276
Haemoglobin (g/L)	Pearson Correlation	0.009
	Sig. (2-tailed)	0.889
	N	262

** .Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table-2: Diagnostic performance of glycated haemoglobin (HbA1c) at 5.36% against the gold standard results of 2-hour oral glucose tolerance test (OGTT) after 75-grams glucose load test. (n=280).

Diagnostic performance HbA1c at 5.36%				
		GDM	GDM	
		Diagnosed	not Diagnosed	
Diagnosis of GDM based upon the results of 2-hour OGTT	Positive	35	103	PPV (25.36%)
	Negative	15	127	NPV (89.44%)
		Sensitivity (70%)	Specificity (55.22%)	Efficiency (57.86%)

GDM: Gestational diabetes mellitus, PPV: Positive Predictive Value, NPV: Negative Predictive Value

study. Mean pregnancy age at the time of presentation was 28.26±4.83 years and mean week of pregnancy was 21.43±6.37. Parity ranged from 1 to 9, with 63(22.5%) showing parity 2, 59(21.07%) parity 1, 59(21.07%) parity 3, 45(16.07%) parity 4, 25(8.93%) parity 5, 14(5%) parity 6, and 15(5.36%) having parity status >6. Mean parity was

Table-3: Diagnostic performance of glycated haemoglobin (HbA1c) at 6.06% against the gold standard results of 2-hour oral glucose tolerance test (OGTT) after 75-grams glucose load test. (n=280).

Diagnostic performance HbA1c at 6.06%				
		GDM	GDM	
		Diagnosed	not Diagnosed	
Diagnosis of GDM based upon the results of 2-hour OGTT	Positive	19	35	PPV (38.78%)
	Negative	31	195	NPV (86.28%)
		Sensitivity (38%)	Specificity (84.78%)	Efficiency (72.86%)

GDM: Gestational diabetes mellitus.

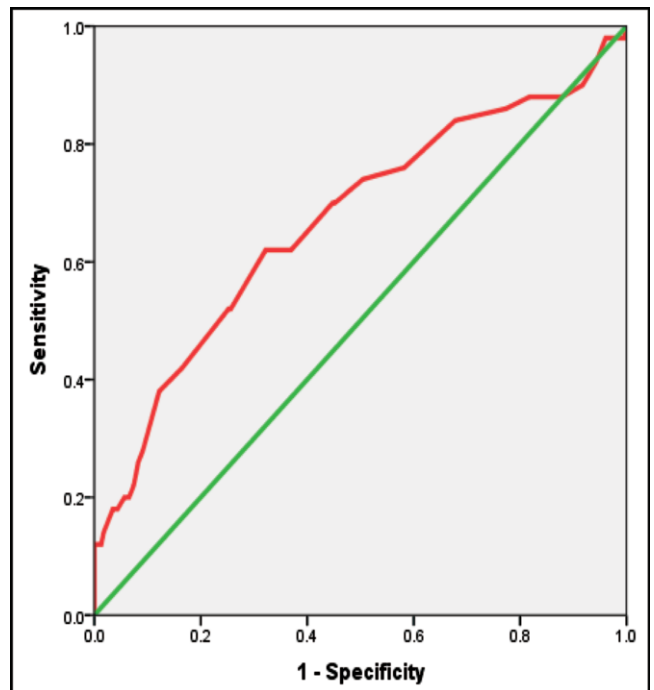


Figure-2: Area Under curve (AUC) for glycated haemoglobin (HbA1c) results considering the results of International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria using 2-hour oral glucose tolerance test (OGTT) with 75 grams glucose load (n=280), p<0.001.

3.08±1.77. Also, 81(28.93%) females had no children, 91(32.5%) had 1 child, 54(19.29%) had 2 children, 36(12.86%) had 3 children, 14(5%) had 4 children and 4(1.43%) had >4 children. The relevant mean value was 1.36±1.25. Of the total, 251(89.64%) subjects had no history of abortion/miscarriage, 18(6.43%) had 1 abortion, 2(0.71%) had 2 abortions, 1(0.36%) had 3 abortions, 4(1.43%) had 4 abortions and 1(0.36%) subject had 5 abortions. Data was not available for 3(1.07%) subjects. Mean abortion was 0.16±0.65. There were 59(21.07%) nulliparous women. Out of the remaining 221(78.93%) females, 47(21.27%) had one operative delivery,

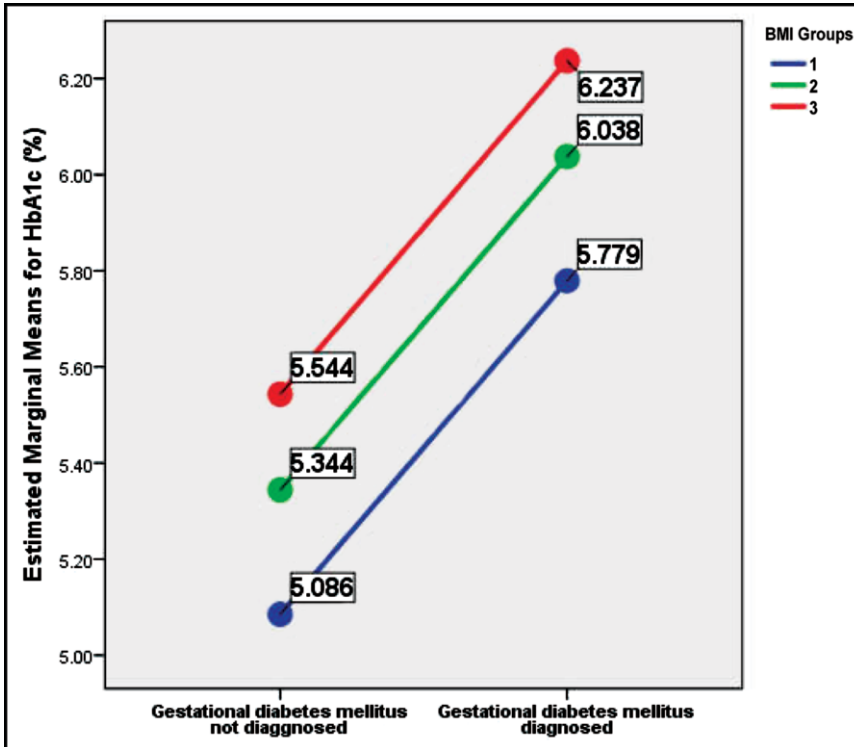


Figure-3: General linear model (GLM) where the effect of body mass index (BMI) as a covariate being depicted along with gestational diabetes mellitus (GDM) diagnosis being kept as an independent factor. (Model significance: $p < 0.001$).

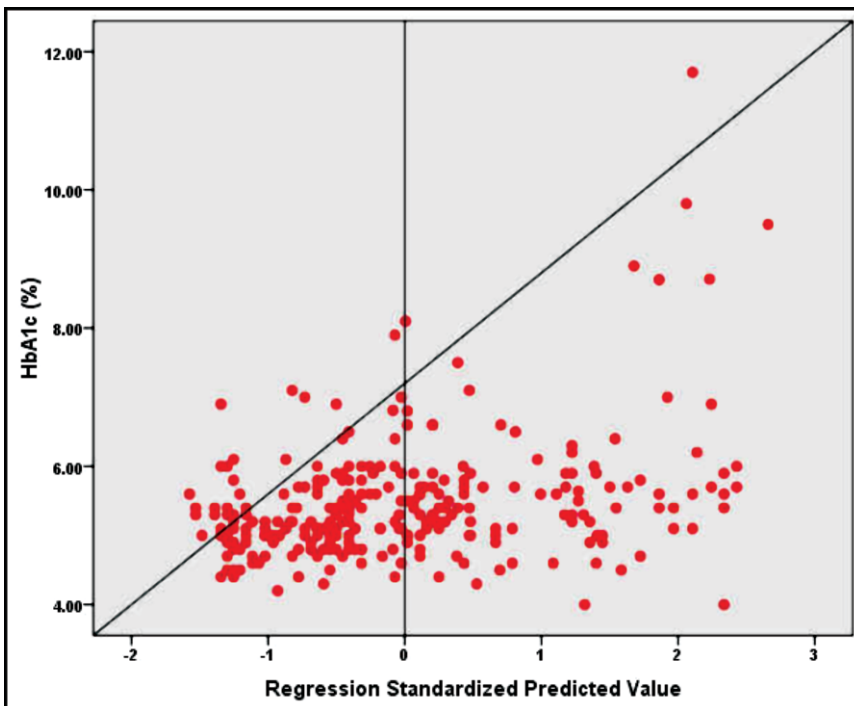


Figure-4: Regression analysis to analyse the contribution of from independent variables including hyperglycaemia as detected by International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria based diagnosis, body mass index (BMI) and age on glycated haemoglobin (HbA1c) (Dependent variable).

29(13.12%) had 2 operative deliveries, and 18(8.14%) had >3 operative deliveries. GDM was found in 50(17.85%) subjects who comprised group A. Mean HbA1c level was higher in this group compared to group B (Figure-1). There was a moderate positive linear correlation for plasma glucose measures and weak positive linear correlation for age and BMI with HbA1c (Table-1).

ROC curve analysis showed AUC as 0.668 (95% confidence interval [CI]: 0.578-0.759; $p < 0.001$; Figure-2). The diagnostic performance of HbA1c with the selected cut-off values of 5.36% and 6.06% was evaluated in the two groups (Tables-2, 3). GLM showed increasing glycation in group A subjects with worsening effect of increasing BMI. The effect of BMI seemed to be independent of GDM diagnosis in pregnant subjects (Figure-3). Similarly, the process of ageing also seemed to add to HbA1c in pregnant subjects ($p = 0.019$). In regression analysis, one unit increase in independent variables, like GDM diagnosis, BMI and age, there was 0.307%, 0.184% and 0.014% increase in HbA1c% respectively (Figure-4).

Discussion

The current study is a pioneer regional study in terms of directly evaluating HbA1c as a diagnostic tool for diagnosing GDM in mid-pregnancy. The study was able to demonstrate significantly higher HbA1c values among GDM subjects as per IADPSG criteria.⁶ This finding is in accordance with data by Khan R et al. and augments the data by Shobha P et al. and Bhavadharini B et al. where they were able to associate adverse pregnancy and foetal outcomes with higher glycation values.¹⁵⁻¹⁷ Literature review is replete with multiple studies having results in contrast to our findings.¹⁸⁻²² Though multiple possibilities could be considered for these variations, like the subject selection criteria, race, regional effects and methodology of

HbA1c,^{23,17,24} we feel that our NGSP-certified HbA1c technique highlighted differences are important. Regional and racial differences suggest an emerging pandemic of metabolic disorders, including DM in sub-continental population where haemoglobin glycation indices are more varied than from the rest of the world.²³ Another possibility could be the fact that diabetic phenotype among our population may be more susceptible to GDM and related pregnancy-related complications, as depicted by studies.^{25,26} Furthermore, there is also evidence to support our findings from other parts of the globe.^{27,28}

The focal question arises as to whether these significant differences in HbA1c results can be translated for HbA1c to be counted as a clinical marker to diagnose GDM in our population to avoid time-consuming, labour-intensive, fasting-based 2-hour OGTT which many patients were not even able to complete due to multiple reasons. We believe that the two highlighted cut-offs of 5.36% and 6.06% for HbA1c can actually help in the diagnosis of GDM. Currently, glycaemia among non-pregnant subjects have been classified as normoglycaemia, impaired fasting glucose (IFG) and diabetes. A similar pattern can be utilised for GDM, where the lower cut-off of 5.36, having a sensitivity of 70%, can be utilised to rule out GDM and the higher cut-off of 6.06 having a specificity of 84.78% can help in confirming the GDM diagnosis. Similar approaches have been recommended in recent literature.^{29,30}

Most metabolic diseases are multi-factorial in terms of causation factors and not limited to one aetiology in pathogenesis and so, evaluating just one factor cannot provide a wholesome view about the disease. The current study highlighted glycaemic status, BMI and, to some extent, age as contributing factors towards the build-up of HbA1c. This finding is in concordance with the results of Capula C et al. and Hoorsan H et al.^{31,32} We, therefore also feel that any lab workup to diagnose GDM must also include BMI and age and, therefore, index of suspicion for diagnosing GDM may be lower in subjects with a higher BMI or age >35 years.

The current study has certain limitations. The most important limitation is its less-powered status and cross-sectional design which has thrown up more questions rather than resolving the diagnostic dilemma surrounding GDM. We recommend that a controlled, multi-central trial must follow the current study to validate our findings.

Conclusion

HbA1c provided a sensitivity of 70% at the lower cut-off 5.36% and specificity of 84.78% at 6.06% where the

former cut-off can help rule out a diagnosis of GDM <5.36% and thus avoid 2-hour OGTT test. The later cut-off of 6.06% can rule in GDM.

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Conflict of Interest: None.

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