

Estimation of Glycated Hemoglobin A (HbA1c) at Time of Pregnancy Diagnosis as A Discriminative Early Test for Women Vulnerable to Develop Gestational Diabetes Mellitus at the 24th Gestational Week

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ABSTRACT

Aim: Estimation of blood glycated hemoglobin A (HbA1c) level in newly pregnant women at time of pregnancy diagnosis (T0) and 3-monthly thereafter to detect any relation between these levels and the change in maternal blood glucose (BG) levels during pregnancy.

Material and Methods: 304 newly pregnant women gave fasting blood samples for estimation of T0 HbA1c level and then underwent the 75-Oral glucose tolerance test (OGTT) and were asked to re-attend the clinic overnight fasting at the start of the 12th and at the 24th-28th gestational week (GW) for estimation of HbA1c levels and to repeat the 75-OGTT. Δ HbA1c was calculated as the difference between HbA1c levels estimated at 12th GW minus T0 levels. The results of the 75-OGTT were interpreted for diagnosis of gestational diabetes mellitus (GDM) and HbA1c at range of 4-6% indicates non-diabetic state. Study outcome is the ability of T0 levels of HbA1c to discriminate women vulnerable to develop GDM around the 24th to the 28th GW.

Results: 38 women developed GDM, while 286 women completed their pregnancy free of GDM. GDM women had significantly higher T0 body mass index (BMI) and HbA1c levels than Non-GDM women. Moreover, the 12th and 24th GW HbA1c levels and Δ HbA1c were significantly higher in GDM women. The 24th GW 2hr-postprandial BG (PPBG) levels showed significant positive correlation with T0 BMI, fasting BG and 2hr-PPBG and with T0 and 12th GW levels of HbA1c and Δ HbA1c. Regression analysis defined T0 level of HbA1c, Δ HbA1c, 12th GW HbA1c level and T0 BMI as the significant positive predictor for the 24th GW 2hr-PPBG level. ROC curve analysis defined T0 level of HbA1c and Δ HbA1c as the significant predictors for the 24th GW 2hr-PPBG level which is diagnostic for GDM.

Conclusion: Development and severity of GDM could be predicted at time of pregnancy diagnosis by high HbA1c level and assured by calculation of the extent of change in HbA1c level at the 12th GW.

Key Words: Early predictors, gestational diabetes mellitus, glycated hemoglobin A

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INTRODUCTION

Glycation of biological macromolecules secondary to hyperglycemia leads to the formation of advanced glycation end products, a reaction which is accelerated in diabetes mellitus^[1]. Glycated hemoglobin (Hb A1c) is the conjugate acid of the Schiff base which develops through a nonenzymatic glycation reaction of human hemoglobin type A^[2]. The reaction consists of initial non-covalent, reversible steps involving glucose and amino acid residues for the formation of labile Hb A1c^[3]. Then, labile Hb A1c undergo slow irreversible formation of stable Hb A1c (the Amadori product), which is measured to assess diabetic progression^[4].

Glucose metabolism during pregnancy is governed by lactogenic hormones stimulating insulin production and counter-regulatory hormones inducing insulin resistance^[5] and disequilibrium between these two forces plays a

major role in pathogenesis of metabolic disorders and carbohydrate metabolism^[6]. Gestational diabetes mellitus (GDM) is defined as glucose tolerance disorder that starts during pregnancy and is associated with increased fetomaternal morbidity^[7]. The International Association of Diabetes in Pregnancy documented that GDM carries a 41.5% and 10.7% risk of maternal prediabetes and type-2 diabetes, respectively after 11.4 years of follow up and is also associated with higher rates of childhood overweight and obesity^[8].

Pathogenesis of GDM was heavily studied but no definite underlying sole mechanism was determined^[9]; disturbed levels of adipokines, which are substances of hormonal character that are secreted by adipose tissue were documented among women who later develop GDM than women completed their pregnancy free of GDM^[10]. Insulin resistance increases during pregnancy which can lead to hyperinsulinemia, GDM, and neonatal hypoglycemia^[11].

Screening of GDM at 24-28 gestational weeks (GW) delays its diagnosis and the implementation of prophylactic methods, thus necessitating improved screening strategies for early discrimination of vulnerable women^[12].

Moreover, international guidelines recommend early screening in the first trimester for overt diabetes and women who have a fasting blood glucose (FBG) levels diagnostic of GDM in later pregnancy are to be identified and treated despite uncertainty regarding the risks and benefits^[13].

AIM OF THE WORK

Estimation of blood HbA1c level in newly pregnant women at time of pregnancy diagnosis and 3-monthly thereafter to detect any relation between these levels and the change in maternal blood glucose levels during pregnancy.

PATIENTS AND METHODS

This is a prospective comparative clinical trial conducted at Department of Obstetrics and Gynecology, Benha University Hospitals and multiple private centers

The current study was conducted according to the conditions of the Local Ethical Committee since October 2018 till July 2020. All pregnant women who attended the Antenatal clinics for assurance of diagnosis of being pregnant and signed written fully informed consent to participate in the study were evaluated for eligibility for study inclusion. Demographic data of women assured to be pregnant were collected and included age, weight and height and baseline clinical and obstetric data. Body mass index (BMI) was calculated in kg/m² as weight (kg)/height (m²)^[14].

Exclusion criteria include manifest DM, previous GDM in multipara women, morbid obesity with BMI>35 kg/m²^[15] and liver, or renal diseases. All pregnant women gave blood samples for estimation of random blood glucose to assure absence of manifest DM.

All women were asked to attend the clinic overnight fasting on the next day to give their baseline (T0) blood samples for estimation of HbA1c level and then underwent the 75-Oral glucose tolerance test (OGTT) which consists of estimation of FBG and postprandial BG (PPBG) levels at one and two hours after taking a 75-gm oral glucose diet. All women were asked to re-attend the clinic overnight fasting during at the start of the 12th and at the 24th-28th gestational week (GW) for estimation of HbA1c levels and to repeat the 75-OGTT. ΔHbA1c was calculated as the difference between HbA1c levels estimated at 12th GW and T0 levels.

Investigations: Venous blood samples (5 ml) were collected from the antecubital vein under complete aseptic conditions and were divided into two parts; the first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ ml blood) to prevent glycolysis for estimation

of blood glucose levels using glucose oxidase method^[16]. While, the second part was collected in EDTA containing tube for HbA1c estimation using Latex Turbidimetry (LINEAR CHEMICALS S.L. Joaquim Costa, Montgat, Barcelona, Spain)^[17].

Interpretation of Results : The results of the 75-OGTT were interpreted for diagnosis of GDM according to the recommendations of the International association of diabetes and pregnancy study groups^[18] as follows: FBG ≥92 mg/dl, 1-h BG ≥180 mg/dl and 2-h BG ≥153 mg/dl. Estimated levels of HbA1c at range of 4-6% indicates non-diabetic state, 6-6.5% indicates goal of control, 6.5-8% indicates good diabetic control and >8% indicates need for interference to achieve control^[19].

Study outcomes : The ability of HbA1c levels estimated at T0 to discriminate women vulnerable to develop GDM around the 24th to the 28th GW. The relation between demographic data and HbA1c and development of GDM.

STATISTICAL ANALYSIS

The obtained data were presented as mean, standard deviation, numbers, percentages, median and interquartile ranges. Results were analyzed using One-way ANOVA for analysis of variance between groups, paired t-test for analysis within each group, Chi-square test (X^2 test) for analysis of non-numeric data and Mann-Whitney test for median values. Sensitivity and specificity of studied parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05 and paired-sample AUC differences was determined versus the null hypothesis that AUC=0. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. *P value* <0.05 was considered statistically significant.

RESULTS

The study included 327 women eligible for evaluation, 23 women were excluded and 304 women were included in the study (Figure 1). Repeated OGTT at the 24th to 28th GW, diagnosed 38 women as having GDM (GDM group) for a frequency of GDM of 12.5%, while the results of OGTT of the remaining 286 women (87.5%) were within normal ranges and were considered as free of GDM (Non-GDM group). Women who developed GDM had significantly higher BMI at time of pregnancy diagnosis, while other demographic and clinical data of studied women showed non-significant differences between these women as being categorized as GDM and Non-GDM groups (Table 1)

Mean FBG and PPBG levels estimated at time of pregnancy diagnosis (T0) were non-significantly (*p*>0.05) higher in GDM women compared to non-GDM women. At the 24th GW, FBG and 2-hr PPBG levels were significantly

higher in all women in comparison to their respective T0 levels and were significantly higher in GDM women compared to corresponding measures of non-GDM women at 24th GW.

Concerning estimated levels of HbA1c at T0 samples mean estimated levels in women who later on developed GDM were significantly higher in comparison to levels estimated in women who continued their pregnancy free of GDM. Similarly, HbA1c levels estimated at 12th and 24th GW were significantly higher in GDM women than in Non-GDM women. Moreover, Δ HbA1c was significantly higher in GDM women than in Non-GDM women at 12th GW (Table 2, Figure 1).

Severity of DGM as judged by 2-hr PPBG levels estimated at 24th GW showed significant positive correlation with BMI ($r=0.165$, $p=0.003$), FBG ($r=0.128$, $p=0.022$) and 2hr-PPBG ($r=0.161$, $p=0.004$) determined at time

of pregnancy diagnosis and showed positive significant correlation with T0 and 12th GW levels of HbA1c and with Δ HbA1c ($r=0.266$, 0.511 and 0.379 ; $p<0.001$)

Regression analysis (Stepwise method) defined HbA1c level estimated at time of pregnancy diagnosis (T0) as the persistently significant positive predictor for 2hr-PPBG level estimated at the 24th GW, followed by Δ HbA1c, HbA1c level estimated at the 12th GW and the T0 BMI, while excluded the other variables as predictors for development or severity of GDM at the 24th GW (Table 3).

ROC curve analysis defined T0 level of HbA1c and Δ HbA1c as the significant predictors for the 2hr-PPBG estimated at the 24th GW which is diagnostic for GDM. However, paired-sample area difference under the ROC curves for T0 level of HbA1c and Δ HbA1c showed non-significant difference (AUC difference= -0.030 ± 0.362 ; $p=0.656$) in favor of T0 level of HbA1c (Table 4, Figure 2).

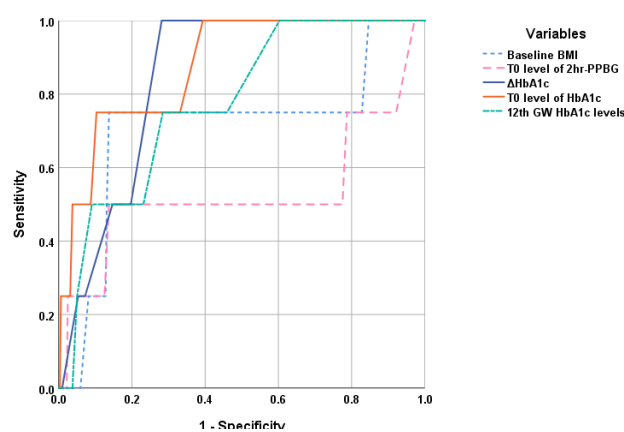


Fig. 1: ROC Curve analysis for predictors of 2hr-PPBG level at the 24th GW

Table 1: Demographic and clinical data determined at time of pregnancy diagnosis

Data	Group	Non-GDM (n=286)	GDM (n=38)	P value
Age (years)		27.9±2.9	28.4±3.2	0.291
BMI data	Weight	83.3±7	86.2±8.7	0.0196*
	Height (cm)	169.7±3.4	170.1±3	0.475
	BMI (kg/m ²)	28.9±2.3	29.8±3.2	0.040*
Obstetric history	Gravidity			
	Primigravida	28 (9.8%)	6 (15.8%)	0.257
	Multigravida	258 (90.2%)	22 (51.3%)	
Parity				
	Primipara	152 (53.1%)	16 (42.1%)	0.201
	Multipara	134 (46.9%)	21 (57.9%)	
Blood pressure	Systolic	117±5.1	117.7±3.5	0.396
	Diastolic	77.9±4.4	77.7±3.8	0.768
Random blood glucose (mg/dl)		89±7.7	90.6±8.2	0.099

Data are presented as mean, standard deviation, numbers & percentages; BMI: Body mass index; *: indicates significant difference

HbA1c FOR WOMEN VULNERABLE TO DEVELOP GDM

Table 2: Results of 75-OGTT and estimation of HbA1c during pregnancy in women of both groups

Variable	Time Group	At T0 time		At 24 th -28 th GW			
		Non-GDM (n=286)	GDM (n=38)	Non-GDM (n=286)	GDM (n=38)		
	FBG	80.3±7	81.5±4.6	81.7±8	101.4±8.5		
	1-hr PPBG	154.5±9.4	156.6±12.3	157.1±10	194.4±32.9		
	2-hr PPBG	124.3±9.5	127.4±9.3	126.1±10.6	163.5±8.6		
75-OGTT	P1		0.313		<0.0001		
			0.217		<0.0001		
	P2		0.061		<0.0001		
				0.03	<0.0001		
			0.022	<0.0001			
	Time Group	At T0		At 12 th GW		At 24 th -28 th GW	
		Non-GDM (n=286)	GDM (n=38)	Non-GDM (n=286)	GDM (n=38)	Non-GDM (n=286)	GDM (n=38)
HbA1c	Level	4.3±0.4	4.5±0.4	4.5±0.3	5.2±0.5	5.2±0.7	7.6±0.75
	P1		0.012		<0.0001		<0.0001
	ΔHbA1c			0.1 (IQR: 0-0.3)	0.65 (IQR: 0.4-0.9)		
	P1				<0.0001		

Data are presented as mean, standard deviation, percentages; GW: Gestational week; T0: Time of pregnancy diagnosis; GDM: Gestational diabetes mellitus; OGTT: Oral glucose tolerance test; HbA1c: Glycated hemoglobin A; ΔHbA1c = HbA1c level at 12th GW minus T0 level; P1 indicates significance of difference between GDM and Non-GDM groups; P2 indicates significance of difference between FAV and at 24-28th GW findings; *P*<0.05 indicates significant difference; *P*>0.05 indicates non-significant difference

Table 3: Standardized Coefficient of included variable for Regression analysis for prediction of the 24th GW 2hr-PPBG level as a measure for severity of GDM diagnosed at the 24th GW

Variables	Model 1		Model 2		Model 3		Model 4	
	β	p	β	p	β	p	β	p
T0 level of HbA1c	0.415	<0.001	0.431	<0.001	0.511	<0.001	0.511	<0.001
ΔHbA1c	0.175	<0.001	0.165	<0.001	0.162	0.001		
12 th GW HbA1c levels	0.149	0.007	0.147	0.009				
Baseline BMI	0.117	0.011						

B: Standardized Coefficient; T0: Time of pregnancy diagnosis; HbA1c: Glycated hemoglobin A; ΔHbA1c = HbA1c level at 12th GW minus T0 level; BMI: Body mass index; P indicates significance of the β value; *P*<0.05 indicates significant difference; *P*>0.05 indicates non-significant difference

Table 4: Standardized Coefficient of included variable for Regression analysis for prediction of the 24th GW 2hr-PPBG level as a measure for severity of GDM diagnosed at the 24th GW

Variables	AUC	Standard error	P value	95% confidence interval
Baseline BMI	0.707	0.158	0.155	0.395-0.999
T0 level of HbA1c	0.876	0.072	0.010	0.735-0.999
T0 level of 2hr-PPBG	0.529	0.200	0.840	0.137-0.922
12 th GW HbA1c levels	0.774	0.099	0.059	0.579-0.969
ΔHbA1c	0.845	0.049	0.018	0.750-0.941

AUC: Area under curve; T0: Time of pregnancy diagnosis; HbA1c: Glycated hemoglobin A; ΔHbA1c = HbA1c level at 12th GW minus T0 level; BMI: Body mass index; GW: Gestational week; P indicates significance of the AUC; *P*<0.05 indicates significant difference; *P*>0.05 indicates non-significant difference

DISCUSSION

Estimated levels of HbA1c showed progressive increase with each estimation in comparison to that estimated at time of pregnancy diagnosis (T0). Moreover, T0 levels of HbA1c were significantly higher in GDM women in comparison to Non-GDM women. Similarly, multiple studies detected significantly higher HbA1c levels in women who developed GDM in comparison

to those who completed their pregnancy free of GDM manifestations^[20,21,22].

Statistical analyses defined high T0 levels of HbA1c and high ΔHbA1c as early specific discriminators for women vulnerable to development of GDM at the 24-26th GW and paired-sample area analysis for AUC for T0 level of HbA1c and ΔHbA1c showed non-significant difference in favor of T0 level of HbA1c, so that T0

level of HbA1c could be considered as the significant early predictor for upcoming GDM, which spares time till the 12th GW before institution of prophylactic and/or therapeutic measures. In line with the results of statistical analyses, Zhuang *et al.*,^[23] using multivariate logistic regression analysis showed that high BMI, 2-h PPBG and HbA1c were significant independent risk factors for GDM. Moreover, the results of a meta-analysis on the screening and diagnostic accuracy of HbA1c test in pregnant women documented that the HbA1c test is simple, quick and more acceptable than the OGTT and can be considered as a specific test at a cut-off of 5.7% with 10% false positive rate^[24]. Also, Immanuel *et al.*,^[25] found HbA1c levels estimated during pregnancy significantly increase across the late GDM, early GDM, overt diabetes and T2DM groups, so can differentiate pregnant women according to pregnancy-imposed glucogenic effect and also found an early HbA1c $\geq 5.7\%$ showed specificity for GDM diagnosis of 89.1%, 88.6% and 89.4%, before 20th GW, at 24-28th GW and throughout gestation, respectively^[26].

ROC curve analysis defined FAV levels and Δ HbA1c are the significant predictors for upcoming GDM, but paired-test analysis for AUC favored FAV levels of HbA1c as a predictor. These findings go in hand with Lia *et al.*,^[27] who found AUC of HbA1c level for detecting GDM was 0.664 and was significant versus the null hypothesis, and HbA1c at $<5\%$ has negative predictive value of 87.9% for possibility of getting diabetic. Also, Battarbee *et al.*,^[22] evaluated HbA1c as a diagnostic test for early GDM compared with OGTT and found the AUC for HbA1c compared with OGTT was 0.80 and the optimal HbA1c threshold was 5.6% as it gives 64% sensitivity, 84% specificity for diagnosis of GDM.

The current study reported that all pregnant women showed a deviation towards hyperglycemia during pregnancy as noticed in women of Non-GDM group whose FBG and 2-hr PPBG levels estimated during 75-OGTT at the 24th GW were significantly higher in comparison to levels estimated during T0 tolerance test. Moreover, this hyperglycemia was exaggerated in some women who developed BG levels on 24-GW tolerance test diagnostic of GDM by a frequency of 12.5%.

These findings point to a fact that pregnancy is a diabetogenic state, but certain women were vulnerable to manifest diabetic BG levels and go in hand with Li *et al.*^[28] who found the levels of FBG, PPBG and insulin resistance scorings were significantly higher in GDM than in normal glucose tolerance women who showed progressive increase of their FBG and PPBG but do not approach the diagnostic level of GDM. Explanation of pathogenesis of gestational glucose intolerance is extremely important, but was still indefinite^[29] and Li *et al.*,^[28] attributed it to progressively declining pancreatic β -cell function as manifested by increased insulin resistance scorings. As another explanation, disturbed levels of

adipokines, which are secreted by adipose tissue, during pregnancy may play an important role in pathogenesis of gestational hyperglycemia, insulin resistance and progression to GDM. In support of such explanation, Francis *et al.*,^[10] studied panel of adipokines; fatty acid binding protein-4, chemerin, and soluble leptin receptor and indicated a role for these adipokines in the pathogenesis of GDM with significant associations detected early in pregnancy before typical GDM screening time. Also, Geça *et al.*,^[29] found serum concentrations of C1q/tumor necrosis factor-related protein-3, one of adipokines, were significantly higher in women with GDM, irrespective of being diet-or insulin treated or not treated at all in comparison to levels estimated in control group.

Interestingly, at time of diagnosis of pregnancy, women who developed GDM were found to have significantly higher BMI than non-GDM women and statistical correlations detected a positive significant correlation between BMI at time of pregnancy diagnosis and severity of GDM as judged by 2-hr PPBG during glucose tolerance testing at 24th GW. In line with these findings, multiple previous studies found women with GDM had higher BMI than women free of GDM^[30,31,32] detected reduced risk of GDM with their proposed diet and exercise interventions for pregnant women versus women received no intervention. Recently, Qiu *et al.*,^[33] reported that for pre-pregnancy BMI, significant differences were found in GDM incidence between the different groups according to BMI and Wang *et al.*,^[34] found women with GDM with a predominant insulin-sensitivity defect had a higher pre-BMI. These findings could be attributed to the fact that plasma concentrations of adipokines, which may contribute directly and/or indirectly for glucose intolerance, are influenced by the amount of adipose tissue present before and/or during the pregnancy^[35].

CONCLUSION

Pregnancy induces maternal hyperglycemic state that may progress to GDM and was related to maternal BMI defined at time of diagnosis of pregnancy. Development and severity of GDM could be predicted at time of pregnancy diagnosis by high HbA1c level and assured by calculation of the extent of change in HbA1c level at the 12th GW.

CONFLICT OF INTERESTS

There are no conflict of interests.

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