

LEVELS IN PREGNANT WOMEN WITH GESTATIONAL DIABETES MELLITUS

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ABSTRACT

Glycated haemoglobin is a form of haemoglobin which is measured primarily to identify the average plasma glucose concentration over a period of 15-17weeks. This study was undertaken to determine the level of HbA1c in GDM patients attending antenatal care clinic in Aminu Kano Teaching Hospital. The verbal consent of 180 pregnant women was obtained. Ninety of them were having gestational diabetes mellitus while the remaining 90 were healthy pregnant women to serve as controls. Blood samples were aseptically collected using venipuncture method. The sera samples obtained were analyzed for fasting blood glucose using the glucose oxidase method. An ion exchange resin method for glycated haemoglobin A1c was employed in the study. An agreed inclusion and exclusion criteria within the scope of this work was also adopted. The average mean of HbA1c levels among women with gestational diabetes mellitus was found to be $5.9\pm 0.32\%$ and in healthy pregnant women as $5.31\pm 0.3\%$. The fasting blood glucose of healthy pregnant women was found to be 4.54 mmol/L and that of pregnant women with GDM was 5.07mmol/L. There is a significant difference in the fasting blood glucose and glycated hemoglobin A1c between the control and the GDM patients with p-value <0.005 .

Keywords: Diabetes mellitus, glycated haemoglobin, Pregnancy, Glucose

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INTRODUCTION

Glycated haemoglobin also referred to as haemoglobin A1c is an important marker in determining how well diabetes is being controlled (11). Haemoglobin is an oxygen carrier throughout the body contained within the erythrocytes. Uncontrolled diabetes mellitus leads sugar build up in blood and combines with haemoglobin becoming glycated. Therefore, the average amount of sugar in the blood can be determined by measuring HbA1c.

Glycated haemoglobin (HbA1c) was initially considered as an “unusual” haemoglobin in patients with diabetes over 40 years ago (11). Correlation between serum glucose and Hb1c levels have been established in the recent past from numerous studies, warranting the use of HbA1c

measurement as an objective index of glycaemic control . A study comprising of 643 participants representing a range of HbA1c levels, validated the relationship between HbA1c and average glucose across a range of diabetes types and patient populations. HbA1c test has become a cornerstone in clinical practice since its introduction in 1980s (1,11). Fructosamine and glycohaemoglobin are both used to monitor diabetic control. However, glycated haemoglobin provides information on the average glucose level over the period of 15 to 17 weeks which reflects the life span of erythrocytes of 120days, thereby indicating a long-term glycaemic control (11). Whereas fructosamine provides average glucose level over the period of 2 to 3weeks, an index intermediate-term diabetic control as opposed to the longer term for glycated haemoglobin (1,11). Also, because of the

shorter life span of the glycated albumin and total proteins, fructosamine measurements are more sensitive to changes in diabetic control. This provides a means to alert the physician to improvement, or deterioration in control much earlier than glycated haemoglobin determinations (5).

HbA1c reflects average plasma glucose over the previous 15 to 17 weeks (5) and measurement of HbA1c can be conducted irrespective of the fasting state of the subject at anytime. This flexibility has made it the preferred test for assessing glycaemic control in people with diabetes and as a screening test for people at high risk of diabetes (11). Glucose intolerance recognized with the onset of pregnancy is considered as gestational diabetes mellitus (WHO 2011). It is often identified at 24th week of gestation and accounting for 7% of cases of diabetes mellitus in pregnancy (7)

High risk complications in pregnancy are seen in people with gestational diabetes mellitus, hence warranting the need for intensive glycaemic control to reduce the risk of intrauterine foetal death, foetal growth disorders and maternal complications (4). In view of the general increase in glycation of many non-enzymatic proteins, other forms of glycated proteins have been considered as indices in monitoring control in diabetic patients (4). Though sensitivity of HBA1C is lower than oral glucose test (OGTT), which remains the golden standard of gestational diabetes, the inconvenience associated with the protocol generates the need to standardize the detection and threshold of HBA1c by establishing the level at different trimesters

Ethical Approval and Consent

The study protocol was approved by Aminu Kano Teaching Hospital Ethical Committee, while informed consent was obtained from subject before blood collection.

Research Protocol

Inclusion criteria: Pregnant women who are Hausa/Fulani

Exclusion criteria: Non Pregnant women

MATERIALS AND METHODS

Thirty (30) pregnant women with gestational diabetes mellitus under each trimester, with mean age of 30 years (range 15 _ 45years) attending antenatal care clinic in Aminu Kano Teaching Hospital were recruited for the study. Thirty healthy pregnant women with mean age of 15 to 45 years, under each trimester attending antenatal care clinic in Aminu Kano teaching hospital were also recruited for the study to serve as controls. Blood sample was obtained from each subject using the veni-puncture procedure. The site of collection (antecubital vein) was swabbed with 70% alcohol in a circular motion and was allowed to air dry. Blood was collected into fluoride oxalate vacutainer in order to obtain plasma for the estimation of glucose, while blood for the estimation of glycated haemoglobin was withdrawn into an EDTA container.

Serum Glucose Estimation (oxidase - peroxidase method) by Barham and Trinder (2)

Principle: Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The oxygen released from the breakdown of hydrogen peroxide by peroxidase enzyme reacts with 4-aminophenazone and phenol to give pink color. Sample, standard and blank, 10µl each was added in test tubes labelled tests, standard and blank respectively. Glucose oxidase reagent, 1000µl was added in each of the labelled test tubes. The above was incubated at 37°C for 10 minutes. The reaction mixture was read at 540nm against reagent blank.

Calculations;

Concentration of glucose = Absorbance of test/absorbance of standard x concentration of standard

Glycated haemoglobin analysis by ion exchange resin method (Trivelli (8))

Principle: Whole blood was mixed with lysing reagent to prepare the haemolysate. This is then mixed with a weakly binding cation exchange resin. The non-glycosylated haemoglobin binds to the resin leaving glycated haemoglobin free in the supernatant. The glycated haemoglobin percentage is determined by measuring the absorbance of the glycated haemoglobin fraction and of the total haemoglobin.

Method:

Step 1: Haemolysate preparation- Lysing reagent, 0.25 cm³ was added in a test tube. Well mixed blood/control, 0.05 cm³ was then added. The above mixture was well mixed and allowed to stand for 5 minutes at room temperature.

Step 2: Glycated hemoglobin separation and assay. The resin tube was incubated in a water bath until it attained the assay temperature (30°C). Haemolysate from step1, 0.5 cm³ was added. A resin separator was placed in the tube so that the rubber sleeve is approximately 3 cm above the resin level, and was mixed well on a vortex mixer continuously for 5 minutes. The resin was allowed to settle at room temperature for 5 minutes and the resin separator was pushed down until the resin was firmly packed. The supernatant was poured directly in a cuvette and the absorbance was measured at 415nm against deionize water.

Step 3: Total haemoglobin assay

5.0ml of deionized water was taken into a test tube. 0.02 cm³ of haemolysate was added to it. The above was well mixed and read at 415nm against deionized water.

Calculations

$Ghb\% = \frac{\text{Absorbance of Ghb}}{\text{Absorbance of Thb}} \times 7.2 \times \text{temperature factor}$

The corresponding HbA1c was deduced from a table provided by the manufacturer.

Statistical Analysis: The data obtained from the study was analyzed using descriptive statistics of mean and standard deviation. Comparative analysis was done among pregnant women and non-pregnant women using unpaired t-test. All statistical analysis was done using Graphpad in Stat computer statistical software package (version 5.00). P-value <0.05 was considered statistically.

Results

A total of 60 pregnant women were involved in this study. Equal numbers were distributed between the patient and the control; of which 30 were gestational diabetes group and 30 were of healthy pregnant women serving as the control group.

Table 1 shows the comparison of fasting blood glucose and glycated haemoglobin A1c of the healthy pregnant women (control) and pregnant women with gestational diabetes mellitus.

Table 1: Fasting blood glucose and glycated haemoglobin of pregnant women with gestational diabetes mellitus and healthy pregnant women

Variables	Sample size	Gestational Diabetic Group	Control Group	t-value	p-value
Fasting Blood Glucose (mmo/L)	90	5.07±0.44	4.54±0.52	4.262	0.000**
Glycosylated Haemoglobin HbA1c (%)	90	5.91±0.31	5.31±0.31	7.496	0.000**

Result of mean+std value= **=significant p<0.05 of Diabetic compared control

The table above shows the comparison of the mean and standard deviation of the fasting blood glucose and glycated haemoglobin A1c of the healthy pregnant women (control) and pregnant women with gestational diabetes mellitus. The mean and standard deviation of fasting blood glucose of pregnant women with gestational diabetes mellitus and healthy

pregnant women were 5.07±0.44 mmol/L and 4.54±0.52 mmol/L (p<0001) respectively, while glycated haemoglobin A1c of pregnant women with gestational diabetes mellitus and healthy pregnant women were 5.91±0.31 and 5.31±0.31(0.0001) respectively.

Table 2: Correlation between Trimester and glycated hemoglobin of the GDM patients

Gestation period	Gestational Diabetic Group	Control Group	Correlation with Gestational period	P value
First Trimester	5.80±0.11	5.24±0.21	0.189	0.318
Second Trimester	5.98±0.21	5.33±0.22		
Third Trimester	6.02±0.42	5.41±0.17		

Table 2 shows the correlation between gestational period and glycated haemoglobin of pregnant women with gestational diabetes mellitus with correlation coefficient value of 0.189 and p-value of 0.318. The result shows no correlation between the age and glycated haemoglobin in the GDM patients.

Discussion

Gestational diabetes mellitus is the most common medical complication and metabolic disorder of pregnancy. It occurs in 2.4-22% among the Indian population depending on the socioeconomic status, dietary habit and environment factors (7). The pregnant women in this study were already confirmed to have gestational diabetes using OGTT before they were being enrolled in the study. The fasting blood glucose concentration of pregnant women with gestational diabetes in this study was found to be 5.07 ± 0.44 mmol/L, closer to the result obtained by Metzgar et al. (6) of ≥ 5.8 mmol/L. Monitoring of glucose level and other indices to determine control of blood is important in order to prevent complications such as foetal demise and those which put mother at risk of (3).

Increase in fasting blood glucose and HbA1c in pregnancy has been well noted in previous studies (9). The increase of the two diagnostic indicators in DM is as a result to resistance in glucose utilization under such physiological condition (9). In the recent past Vijayam et al. (10) made similar observation. Since the glycated haemoglobin correlates well with the fasting blood glucose levels, it can be used in the evaluation of long-term glycaemic control.

Though reference range for HbA1c has been set at $>6\%$ (11), it is pertinent to note there have been few studies which looked at the normal reference range of HbA1c in pregnant women and in most cases rely on the and the normal reference range of HbA1c obtained

from non-pregnant subjects to interpret the degree of glycosylation in pregnant women. Furthermore, those studies conducted in pregnancy were done in the third trimester (12). Changes in the level of HbA1c has been noticed over time in pregnancy, thus warrants the need determine the range separately from the two group of women under different physiological condition (12). This approach may be of great assistance in early detection of abnormality and improving the outcome of the pregnancy (12)

When compared with non-pregnant women, HbA1c in pregnant women changes over time. Thus, it is better to define the reference range of HbA1c in healthy pregnant women and pregnant women with glucose intolerance which may be helpful for the improvement of pregnancy outcome. Glycated albumin is known to have shorter half live than HbA1c, hence considered a better index of monitoring protein glycation. It is noteworthy that HbA1c seems to be consistently elevated, but not glycated proteins in some pregnancy due to deficiency of iron (4). Therefore, Iron deficiency may be a hindrance in giving glycated albumin advantage as a diagnostic tool in assessment of glucose utilization over HbA1

Though oral glucose tolerance test (OGTT) test is known to have higher sensitivity than HbA1c, as diagnostic procedure, in pregnancy its usefulness hindered by reproducibility of glucose measurement due to certain analytical variables, different genetic background of the population and threshold (Yu et al., 2014). Furthermore, normal OGTT may not always exclude GDM, while false-negative rate of the OGTT cannot be accurately calculated (Yu et al., 2014). Though this study has made attempt to address one of the shortcomings of using HbA1c as a diagnostic protocol in the assessment of GDM, further work is required

for its standardization and establishment of threshold. Such effort would increase the therapeutic efficacy of HbA1c test in pregnancy.

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