Glycosylated hemoglobin values in nondiabetic pregnant women in the third trimester and adverse fetal outcomes: An observational study

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ABSTRACT

Objective: The objective of the study is to estimate the level of glycosylated hemoglobin (HbA1c) for a safe fetal outcome and to estimate the relation between this level and various adverse fetal outcomes. Materials and Methodology: Primigravidas who are diagnosed as not having gestational diabetes mellitus as per the glucose challenge test done at 24 weeks with a cutoff value up to 140 mg/dl are followed up at 30–34 weeks for the estimation of HbA1c in the blood and further till the time of delivery and postnatal period for the fetal outcomes. Data were collected based on detailed patient interview, clinical examination, and laboratory investigations. Data were analyzed to obtain the mean value of HbA1c in the third trimester. Fetal outcomes were analyzed with the HbA1c value using Chi-square test. Results: The HbA1c values in the third trimester of pregnancy in this study ranged from 4.5% to 6%. Discussion: Unfavorable outcomes were found the least in the 4.5%–5%. The average plasma blood glucose corresponding to HbA1c value of 5% is 101 mg/dl. The majority of the newborn were admitted for observation for transient tachypnea (49.5%), hypocalcemia requiring calcium supplements (12.6%), hypoglycemia requiring glucose (7.8%), and persistent tachypnea of newborn (5.8%) and all the outcomes correlated significantly with HbA1c values. Conclusion: Hence, HbA1c can be utilized for the monitoring of glycemic level and as screening test.

Keywords: Fetal outcomes, glycosylated hemoglobin, nonpregnant diabetic women, third trimester

Introduction

The diabetogenic potential of pregnancy has been studied as early as 1882.¹ There is greater postprandial increase in circulating glucose with decrease in fasting glucose in late pregnancy with increased insulin level with progressive increase in maternal hormones such as progesterone, human chorionic somatomammotrophin (HCS), and human placental lactogen (HPL). These increase the responsiveness of the pancreatic islet cells in late pregnancy, thus increasing the insulin secretion and at the same time altering the insulin sensitivity in target organs. Progesterone affects metabolic processes such as gluconeogenesis.² The rate of maternal to fetal glucose transfer is a function of transplacental concentration gradient. HPL increases the gradient by increasing maternal blood glucose concentration and decreasing the fetal glucose concentration. All these factors promote transplacental movement of glucose.³ The glucose tolerance deteriorates in human pregnancy and is related to a pronounced peripheral resistance to insulin and is caused by postinsulin receptor events probably brought about by the cellular effects of the increased plasma levels of one or more of the pregnancy-associated hormones and free cortisol.⁴ The resistance is mainly confined to the muscle tissue, where significant reductions in the activities of phosphofructokinase and pyruvate kinase involved in glucose metabolism have been demonstrated, which along with increased muscle tissue in pregnancy, leads to a decrease in glycolysis in muscle.⁵–⁷ There was 65% increase in fasting insulin level in late gestation with...
30% increase in basal endogenous glucose in 34–36 weeks of gestation.\textsuperscript{9} The contribution of gluconeogenesis to total glucose was found to be 76% in late gestation (at 34 weeks).\textsuperscript{10} The anti-insulinogenic and lipolytic activities of HCS, prolactin, cortisol, and glucagon, all of whose concentrations increased in the late gestation, contributed to the glucose intolerance, insulin resistance, and adipose tissue mobilization. The insulin resistance, as shown by insulin-glucose ratio, was higher at 37 weeks of gestation and less during postpartum, and this was consistent with the fact that there is persistent glucose production in fasting during pregnancy despite lower fasting glucose concentration.\textsuperscript{10} The change in carbohydrate and lipid metabolism in pregnancy is to ensure a continuous supply of nutrients to the fetus, even though mother takes food intermittently - these changes progress throughout pregnancy. There is fasting hypoglycemia due to fetal consumption, postprandial hyperglycemia, and hyperinsulinemia due to anti-insulin factors. This response is consistent with pregnancy-induced state of peripheral insulin resistance, the purpose of which is to ensure a sustained supply of glucose to fetus to meet its growth demand. Hyperinsulinemia and insulin resistance are most marked in the third trimester of pregnancy.\textsuperscript{11} Progesterone, prolactin, and HPL directly or indirectly mediate the insulin resistance.\textsuperscript{2,11} Certain factors secreted mainly by adipocytes called adipokines include leptin, adiponectin, tumor necrosis factor-alpha (TNF-alpha), interleukin-6, resistin, etc., TNF-alpha is a cytokine produced by various cells such as monocytes, macrophages, neutrophils, adipocytes, and fibroblasts.\textsuperscript{12} TNF-alpha impairs insulin signaling by increasing serum phosphorylation of insulin receptor substrate-1 and decreasing insulin receptor tyrosine kinase activity and is definitively correlated with the insulin resistance.\textsuperscript{13} The changes in insulin sensitivity from early gestation (22–24 weeks) to late gestation (34–36 weeks), correlated with plasma TNF-alpha.\textsuperscript{14}

Pregnancy is a leptin-resistant state as it was seen that in spite of increased leptin levels, which would cause decreased appetite and energy intake, there was increased maternal energy intake with positive energy balance; hence, leptin resistance exists in pregnancy. In addition, the variations in maternal insulin sensitivity are partly due to maternal adipocyte and growth hormone-like growth factor axes.\textsuperscript{15} Specific glucose transporter proteins help in transporting glucose across the placenta down the concentration gradient in normal non-diabetic pregnancy.\textsuperscript{16}

Macrosomia, hypoglycemia (18.4%), respiratory distress syndrome (5.2%), and hyperbilirubinemia (15.3%) were found to be the most common perinatal problems in diabetic pregnancy. The average gestational age was 34–36 weeks in the patients studied.\textsuperscript{17}

Increased pregravid body mass index (BMI) and summed skinfold thickness (triceps plus subscapular) were associated with higher glucose concentrations at first visit and at 28 weeks. Higher maternal glucose was associated with increased infant birth weight. Comparing women whose plasma glucose concentrations were <99 mg/dl with the others gravidas whose glucose concentrations were 99–130 mg/dl and those with glucose concentrations of >130 mg/dl, indicated that birth weights were approximately 50 g higher (P < 0.05) for gravida whose glucose concentrations were 99–130 mg/dl and 200 g higher (P < 0.005) for those with glucose concentrations of >130 mg/dl. The difference in birth weight for women whose glucose concentrations were >130 mg/dl versus above the median was 140 g (P < 0.05). Increasing maternal plasma glucose concentration was associated with decreasing duration of gestation. High glucose concentrations are a risk factor or a risk marker for the subclinical infection that gives rise to choioamnionitis. With increasing BMI, gravida who are not diabetic, have higher glucose concentrations on the standard 50 g screening test.\textsuperscript{18}

Glucose challenge test is an acceptable screening test in which plasma glucose is measured 1 h after ingestion of 50 mg of pure glucose in 150 ml of fluid, to screen for gestational diabetes at 24–28 weeks of pregnancy using a threshold of 140 mg/dl.\textsuperscript{19}

Glucose only is the glycosylating agent and that may be the reason for increase in the level of HbA1c in diabetics and that the aldehyde of glucose and the amino group of valylhistidine can form a reversible Schiff base linkage.\textsuperscript{20,21} The red blood cell is anucleated cell and hence it cannot synthesize hemoglobin (Hb) and all the proteins including Hb is formed while it is in the bone marrow. The glycosylated hemoglobin (HbA1c) levels increased in the lifespan of the cell and that the HbA1c levels increased 2.8 times faster in diabetic than in normal mice.\textsuperscript{22}

The formation of HbA1c is a posttranslational modification and that since it is a very slow process, it is most likely to be nonenzymatic and an example of Maillard reaction (nonenzymatic glycation).

HbA1c, the most abundant minor Hb component in human erythrocytes, is formed by the condensation of glucose with the N-terminal amino groups of Hb A as both glucose and Hb are found in large quantities in the red blood cells – initially an unstable compound is formed, which is followed by the formation of a stable form and the second part of the reaction is irreversible. HbA1c is slowly formed during the 120-day lifespan of the erythrocyte, and the concentration of HbA1c would be higher in older red cells. The amount of HbA1c formed is directly proportional to the amount of glucose concentration in the cell, and hence this reaction is faster in diabetics due to higher glucose concentration, and it may reveal information about adequate control of blood glucose over the period. It forms 4% of total Hb. The concentration of HbA1c was found to be 3%–6% in their study.\textsuperscript{23}

Hb condenses directly and nonenzymatically with glucose to give HbA1c.\textsuperscript{24,25} Glycosylation of Hb is also a slow, nonenzymatic process reflecting the main Hb-A fraction in diabetic patients, and the amount of sugar attached to Hb correlated with average intracellular blood glucose level\textsuperscript{26} and with glycemic levels over the previous 6–10 weeks.\textsuperscript{27,28}
Statistically significant decrease in total HbA1c including HbA1c in healthy pregnant subjects occurred, at about twenty weeks and this decrease was maintained throughout pregnancy and hence its use to assess diabetic control in pregnancy.\textsuperscript{[9]} In a normal pregnancy, between 6 to 10 weeks, there is a decrease in the fasting blood glucose and this continues throughout pregnancy. This reduction in fasting glucose is smaller in subjects with higher BMI.\textsuperscript{[31]} In a study done by, HbA1c values in diabetic pregnancy are lower than in nonpregnant diabetic subjects (mean 7.8% ± 1.6% vs. 9.9% ± 1.9%) and the mean value in pregnant nondiabetic was 4% ± 0.7%.\textsuperscript{[32]}

A decrease in the upper reference limit of HbA1c from 4.7% to 6.3% before pregnancy to 4.5% to 5.7% in early pregnancy and 4.4% to 5.6% in the third trimester occurs. Average HbA1c was found to be 5.1% ± 0.4% in early pregnancy (\(P < 0.001\)) and 5.0% ± 0.3% (\(P < 0.001\)) in late pregnancy. The changes in HbA1c can be attributed to carbohydrate metabolism, changes in erythrocytes or both. Lower concentration of HbA1c facilitates oxygen delivery to the fetus – HbA1c has decreased affinity for 2,3-bisphosphoglycerate and increased affinity for oxygen. New erythrocytes may be exposed to lower average concentration of glucose due to sustained fasting hypoglycemia, with decrease in glycosylation.\textsuperscript{[33]} The formation of HbA1c is a posttranslational modification and that since it is a very slow process, it is most likely to be nonenzymatic and an example of Maillard reaction (non-enzymatic glycation) and HbA1c can be used as a reliable marker for the assessment of glycemic control with periodic monitoring being useful in diabetic patients with the upper range of HbA1c increasing from 5% in first trimester to 5.9% in third trimester.\textsuperscript{[34,35]} The glycosylated haemoglobin correlates best with the mean plasma glucose and urinary glucose levels over previous four to six weeks.\textsuperscript{[36,37]}

An Indian study has shown the mean HbA1c in pregnant Indian women with normal glucose tolerance to be 5.36+/−0.36%.\textsuperscript{[38]} The average plasma blood glucose corresponding to HbA1c value of 5% is 101 mg/dl and that corresponding to mean value of 5.29% is 111 mg/dl (range for 5.29%+/−0.514% is 90 mg/dl to 129 mg/dl).\textsuperscript{[39]}

In view of these maternal changes during a normal pregnancy in glucose levels, which increase as pregnancy progresses, and its effects and that HbA1c correlates with the glycemic control over the past few weeks, the present study aims to estimate the levels of HbA1c in pregnant nondiabetic women in the third trimester and its relation to adverse fetal outcomes.

### Materials and Methodology

The study was conducted for dissertation purpose at Lourdes Hospital, Ernakulam which is a 750 bedded multispecialty referral hospital catering to both urban and semi-urban population. The study is a unicentric, prospective, observational study and was conducted among the outpatient and inpatient pregnant women, in their third trimester that is from 24 weeks onward, attending the Department of Obstetrics and Gynaecology in Lourdes Hospital, Pachalam, Kochi, Kerala.

The duration of the study was March 2013 to March 2014.

Data were collected with the help of a structured questionnaire by the researcher herself, from primigravida subjects in their third trimester at the nursing station near the outpatient department (OPD). It included physical examination and laboratory investigations. The patients were followed up further in the OPD and at the time of admission in the labor room. After delivery, the newborn and the mother were followed up for various outcomes.

Data were analyzed to obtain the mean value of HbA1c in the third trimester. Fetal outcomes were analyzed with the HbA1c value. The collected data were analyzed to arrive at mean values ± standard deviation, for the HbA1c value. The statistical techniques employed were mainly Chi-square test - \(P < 0.05\) was considered statistically significant. Graphical representations in the form of tabulations, bar diagrams, and pie charts were also utilized.

The exclusion criteria were abnormal GCT value more than 140 mg, BMI >25 kg/m\(^2\), previous history of diabetes mellitus, on medications such as oral hypoglycemic agent, steroids, history of iron deficiency anemia, hemolytic anemia, hemoglobinopathies and other conditions such as uremia.

### Results

This study included 100 primigravida women in their third trimester. They belonged to the age group 21–38 years. The mean age was 26.17 ± 3.72 years. The gestational ages at the time of admission ranged from 37 weeks to term, and there were no preterm or postterm subjects. The mean gestational age is 38 weeks 9 ± 2 days.

The prepregnant BMI [Figure 1] varied from 18.2 to 24.8 kg/m\(^2\), the mean value being 21.56 ± 1.37 kg/m\(^2\). A significant association was found between the prepregnant BMI and HbA1c (Chi-square value = 30.067, \(P < 0.001\)).

The weight gain [Figure 2] of the subjects ranged from 8 to 16 kg with the mean value being 11 ± 1.922 kg. HbA1c was 4% ± 0.7% in the prepregnant BMI group and was found to be 5% ± 0.4% in early pregnancy and 5.7% ± 0.5% in late pregnancy. The changes in HbA1c can be attributed to carbohydrate and other conditions such as uremia.

| BMI Category | Subjects |
|--------------|----------|
| <18.5        | 20       |
| 18.5–24.99   | 80       |
| ≥25.0        | 10       |

![Figure 1](image_url)

**Figure 1:** Pie diagram showing the distribution of body mass index.
seen to correlate significantly with the weight gain (Chi-square value = 25.225, P < 0.001).

The mean value of GCT is 118.07 ± 15.219 mg/dl, and the GCT of the second trimester correlated significantly with HbA1c of the third trimester (P < 0.05). The random blood sugar in the third trimester ranged from 85 to 148 mg/dl. The mean value was 124 ± 16.277 mg/dl. There was a significant association between the random blood sugar values and HbA1c, both taken in the third trimester (P < 0.05).

The HbA1c values [Figure 3] in the third trimester of pregnancy in this study ranged from 4.5% to 6%. The mean value was 5.29% ± 0.514%.

There were 54 normal deliveries and 45 cesarean sections with one venticouse delivery. There were in total 96 singleton deliveries, and 4 twin deliveries but one pair of twins had an intrauterine death. Hence, there were in total 103 newborns. The indications for the cesarean sections varied from failed trial, fetal distress, meconium-stained liquor, premature rupture of membranes (one case) and placenta previa (one case), corrected mitral stenosis (elective). Nineteen sections were for failed trial only, 4 were for failed trial and fetal distress. Four sections were done for failed trial, fetal distress, and breech. Four patients underwent cesarean sections for fetal distress and meconium-stained liquor.

The birth weight ranged from 1930 to 3750 g with the mean birth weight being 3140 ± 367 g. A significant association was found between the birth weight and HbA1c value (P < 0.05). Admission to neonatal Intensive Care Unit (ICU) was also found to correlate significantly with HbA1c (P < 0.05). Unfavorable outcomes were found the least in the 4.5%–5% range; hence, the upper cutoff of safe range can be taken as 5%, association with HbA1c values and adverse outcomes being significant (P < 0.05).

The various outcomes [Figure 4] in the newborn analyzed revealed that the majority were admitted for observation for transient tachypnea (49.5%) and hyperbilirubinemia (16.5%) requiring phototherapy, hypocalcemia requiring calcium supplements (12.6%), hypoglycemia requiring glucose (7.8%), and persistant tachypnea of newborn (5.8%). A few newborn required management such as intravenous antibiotics also. All the fetal outcomes correlated significantly with the HbA1c value [P < 0.05, Table 1].

**Discussion**

The study on primigravida in their third trimester who were nondiabetic was found to have a mean HbA1c value of 5.29% ± 0.514% which was in fair agreement with other similar studies. HbA1c values ranged from 4.5% to 6%. The range of HbA1c which had favorable outcome was 4.5%–5%. Hence, we may conclude that the upper cutoff of HbA1c for a safe outcome is 5% for a nondiabetic pregnancy so that adverse outcomes can be minimized. The HbA1c value was significantly associated with weight gain during pregnancy and prepregnant BMI, but no significant association was seen with prepregnant weight. For a female with normal BMI before pregnancy, weight gain of 10–13 kg is recommended. As per a previous study, gain of >13 kg is associated with 9% perinatal mortality in females with <68 kg and 14% perinatal mortality in >68 kg prepregnant weight and gestational age, pregravid weight, weight gain, and BMI all affect birth weight. There was association of HbA1c in the third trimester with GCT done in the third trimester with GCT done in the second trimester and random blood sugar in the third trimester, which were both significant. Similarly, a significant association was found between the birth weight of the newborn, fetal outcomes such as observation in the ICU for transient tachypnea, hyperbilirubinemia, hypocalcemia, and hypoglycemia. It is known that, in pregnant women, who are not overtly diabetic, association is found between increasing glucose levels and fetal outcomes such as birth weight, need for...
intensive care, hypoglycemia, and hyperbilirubinemia as also with obstetrical outcome like cesarean sections.\[41\]

In addition, neonatal hypoglycemia is increased in gestational diabetes patients due to fetal hyperinsulinemia, decreased hepatic glucose production, and decreased ability to use glycogen in the first few days of life. Poor glycemic control in diabetic patient affects lung maturity and hence there is increased risk of respiratory distress syndrome. Hyperbilirubinemia occurs probably due to decreased clearance of bilirubin and increased production of breakdown products as there is associated polycythemia in response to chronic fetal hypoxia. Fetal hypocalcemia can occur due to decreased maternal parathyroid hormone and the severity of hypocalcemia correlates with degree of glycemic control.\[42\]

The average plasma blood glucose corresponding to HbA1c value of 5% is 101 mg/dl and that corresponding to mean value of 5.29% is 111 mg/dl (range for ± 0.514% is 90–129 mg/dl).\[39\]

Hence, it may be concluded that HbA1c can indeed be utilized for the monitoring of glycemic level, as a screening test, even in nondiabetic pregnancies in our clinical practice.

**Recommendations**
- The HbA1c value should be kept between 4.5% and 5% for minimal adverse outcomes it may well be useful to keep the blood sugar values, irrespective of fasting or postprandial levels, to below 101 mg/dl, in the third trimester
- There is a need to keep the BMI also in the lower range since HbA1c is found to increase with the BMI and weight gain, which will require lifestyle management in the preconception period itself
- More frequent measurements of HbA1c beginning from the prepregnant state to the first, second, and third trimesters in our routine clinical practice is required so that the glycemic changes which occur throughout pregnancy, with follow-up into postnatal period, can be monitored.

**Strengths of the study**
- The study is carried out during pregnancy, which is a very vulnerable period, but with minimum risks to the subjects
- This study being a hospital-based study and HbA1c being a common test available in the hospital laboratory, the subjects could get it done with minimum effort.

**Limitations of the study**
- Sample size is small
- Confounding factors such as family history and parity could not be avoided—majority of subjects have history of diabetes mellitus in one or both parents, and primigravida were studied since the incidence of cesarean sections for an indication of previous cesarean sections is already quite high
- Only random blood sugars could be measured as and when the patient came to hospital in the third trimester
- The day of measurement of HbA1c varied as and when the subjects came for their due date of checkup in the third trimester
- Counseling or any other advice was not provided as it is an observational study.

**Conclusions**
Thus, even though GCT may be normal, blood sugars should be measured in the third trimester too, since dietary pattern also changes in the third trimester, due to sociocultural factors, in our society. The average blood plasma glucose corresponding to the mean HbA1c value of 5.29% is 111 mg/dl (range for ± 0.514% is 90–129 mg/dl). The average plasma blood glucose corresponding to HbA1c of 5% is 101 mg/dl. As HbA1c value of 5% is considered to be the safe cutoff in this study, it may well be useful to keep the blood sugar values, irrespective of fasting or postprandial levels, to below 101 mg/dl, in the third trimester.

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**Conflicts of interest**
There are no conflicts of interest.

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