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A Cross-sectional study of glycosylated hemoglobin in different trimesters of normal pregnancy.

Dr. Archana[1], Dr. Anil Kumar Kamal[2], Dr. Ranjit Kumar Arora[3], Dr. Priyanka Shrivastava[4], Dr. Deepak Kumar[5], Dr. Ranjit Kumar Rajak[6], Dr. Madhu Kumari[7]*

[1] *Tutor, Department of Physiology, RIMS, Ranchi[1]*
[2] *Associate Professor, Department of General Surgery, RIMS, Ranchi*
[3] *Professor, Department of Physiology, Palamu Medical College, Daltenganj*
[4] *Associate Professor, Department of Anaesthesiology, RIMS, Ranchi*
[5] *Junior Resident, Department of General Surgery, RIMS, Ranchi*
[6] *Junior Resident, Department of General Surgery, RIMS Ranchi, Jharkhand*
[7] *Senior Resident, Department of Gynaecology, RIMS Ranchi, Jharkhand*

Abstract

Background
Glycated hemoglobin (HbA1c) is widely accepted as a reliable indicator of long-term glycemic control. Unlike oral glucose tolerance tests (OGTT), which are the standard diagnostic tool for gestational diabetes mellitus (GDM) but often poorly tolerated during pregnancy, "HbA1c testing offers a non-fasting, convenient, and stable measure of blood glucose control over the previous 2–3 months. This study aims to assess the physiological variations in HbA1c levels across different trimesters of normal pregnancy in comparison with non-pregnant women of the same age group.

Methods
The study was conducted at the Department of Physiology, RIMS, Ranchi, from January 2016 to February 2017. A total of 60 female subjects, aged between 18 and 30 years, were included: 15 non-pregnant women (controls) and 45 pregnant women, distributed equally across the three trimesters. HbA1c levels were measured using the High-Performance Liquid Chromatography (HPLC) method. Statistical analysis was performed using MedCalc software to determine the significance levels between groups.

Results
The mean HbA1c level in non-pregnant women was 5.55% ± 0.30, which was higher than in all trimesters of pregnancy. In the first, second, and third trimesters, the mean HbA1c levels were 5.12% ± 0.32, 5.33% ± 0.32, and 5.03% ± 0.28, respectively. A significant reduction in HbA1c was observed in the first and third trimesters compared to the non-pregnant group ($p = 0.0007$ and $p < 0.0001$, respectively). The increase in the second trimester was not statistically significant when compared to the first.

Conclusion
HbA1c levels fluctuate during pregnancy due to physiological changes and should be interpreted with trimester-specific references. These findings support the potential utility of HbA1c as a complementary tool for glycemic assessment during pregnancy.

Recommendation
Clinicians consider using trimester-specific HbA1c reference ranges in routine prenatal care to improve monitoring of maternal glycemic status, especially when OGTT is not feasible.

Keywords: *Glycated hemoglobin, Pregnancy, Trimesters, Gestational diabetes mellitus, Glycemic control, Hemodilution, Insulin resistance*
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Corresponding author: *Anil Kumar Kamal[*]*
Email: ***archanakamal1972@gmail.com***
Associate Professor, Department of General Surgery, RIMS, Ranchi

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Introduction

HbA1c is a key biochemical biomarker for chronic glycaemia diagnosis and therapy. Blood glucose levels during the past 8-12 weeks can be estimated by the amount of haemoglobin that has undergone non-enzymatic glycation in the presence of glucose [1]. Because red blood cells have an average lifespan of 120 days, HbA1c captures long-term glycaemic exposure better than single-point fasting or postprandial blood glucose assessments, which can be affected by daily physiological variations, recent food intake, stress, or physical activity [2]. Hemoglobin A1c is more stable and reproducible for clinical surveillance of diabetes mellitus (DM). Due to the increased risk of gestational diabetes mellitus (GDM), which is glucose intolerance of various severity that begins or is first recognised during pregnancy, glucose levels must be regularly monitored [3]. An oral glucose tolerance test (OGTT) between 24 and 28 weeks of pregnancy is the gold standard for detecting gestational diabetes mellitus [4]. However, OGTT has several limitations. It takes a long time, requires fasting, and multiple blood draws, which can cause nausea and vomiting in pregnant women. Due to these limitations, researchers are looking for other screening methods, such as the haemoglobin A1c (HbA1c), a single non-fasting test that measures long-term glucose levels [5].

Despite these benefits, pregnant physiological changes make HbA1c interpretation harder. In addition to glucose metabolism, pregnancy can impact HbA1c levels through hormonal, haematological, and metabolic changes. When plasma volume increases significantly during pregnancy, natural hemodilution occurs [6]. This dilutional effect, together with faster erythropoiesis and red blood cell turnover, may lower HbA1c in the first trimester. Early pregnancy symptoms of hyperemesis gravidarum include decreased oral intake and frequent vomiting [7]. HbA1c levels may drop in the first trimester due to glucose shortages. Hormones fluctuate considerably during pregnancy. Oestrogen, progesterone, placental growth hormone, cortisol, and placental lactogen increase insulin resistance [8]. The body develops insulin resistance to ensure the growing foetus gets enough glucose. Some women develop hyperglycemia and GDM due to insufficient pancreatic beta-cell activity to meet insulin demand [9]. If glucose homeostasis is interrupted, insulin resistance peaks in the second and third trimesters, raising HbA1c. HbA1c concentrations in each trimester are affected by erythrocyte turnover, haemoglobin concentration, iron status, and glycaemic state, making interpretation difficult.

Ethnicity, dietary condition, altitude, iron deficiency anaemia, and other factors can complicate HbA1c findings. HbA1c values may be misinterpreted due to iron deficiency anemia's enhanced glycation and extended erythrocyte lifetime during pregnancy [10]. Due to haemolysis and blood loss during pregnancy and other obstetric issues, erythrocyte survival and HbA1c levels can decrease. HbA1c levels taken during pregnancy, especially for GDM screening or diagnosis, should be interpreted with caution because of these potential confounding variables. Despite these challenges, testing haemoglobin A1c may aid prenatal therapy [11]. Some study suggests that HbA1c cutoff values can predict preeclampsia, macrosomia, and neonatal hypoglycemia. There's no standard HbA1c threshold for GDM diagnosis, and its sensitivity and specificity vary [12].

This study intends to advance understanding by comparing HbA1c values in pregnant and age-matched non-pregnant women. This study, conducted in the Physiology Department at RIMS, Ranchi, enrolls healthy pregnant women throughout the pregnancy. People with diabetes or a family history of the condition and those using erythropoietin or vitamin C, which may alter glycaemic management or erythrocyte turnover, are excluded. HPLC measures HbA1c accurately and consistently.

Materials and methods

Study design and setting

This study was a cross-sectional observational investigation conducted in the Department of Physiology, Rajendra Institute of Medical Sciences (RIMS), Ranchi, over a span of 14 months, from January 2016 to February 2017. The primary objective was to examine trimester-wise variations in glycated hemoglobin (HbA1c) levels among healthy, non-diabetic pregnant women and compare these findings with non-pregnant controls to establish reference ranges.

Study population and sampling

A total of 60 healthy female subjects, aged between 18 and 30 years, were enrolled. These subjects were equally divided into four groups, each comprising 15 participants. The grouping was done as follows:

Group I (Control group): Non-pregnant women with no history of diabetes.
Group II (First trimester): Pregnant women with gestational age up to 12 weeks.

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Group III (Second trimester): Pregnant women between 13 and 28 weeks of gestation.
Group IV (Third trimester): Pregnant women beyond 28 weeks of gestation until term.
Participants were recruited from the Outpatient Department of Obstetrics and Gynecology at RIMS as well as from the population of medical and nursing students within the institution. A purposive sampling technique was employed to ensure representation from all three trimesters of pregnancy.

Inclusion criteria

- Healthy females aged 18–30 years.
- Pregnant participants carrying singleton pregnancies.
- Subjects willing to provide written informed consent and comply with study procedures.

Exclusion criteria

- Known cases or family history of diabetes mellitus.
- Use of medications such as Vitamin C or erythropoietin, which may affect HbA1c values.
- Presence of anemia, as it can lead to altered erythrocyte turnover and influence HbA1c readings.

Blood sample collection and analysis

Every individual gave venous blood samples under aseptic circumstances. We took samples at any time as HbA1c testing doesn't require fasting. After that, the samples were examined in the main lab. We measured HbA1c with HPLC. HPLC measures glycated haemoglobin accurately and reliably, making it the gold standard. The method reduces haemoglobin fluctuations and other misleading biochemical indicators. It was calibrated using the National Glycohemoglobin Standardisation Program (NGSP) and Diabetes Control and Complications Trial (DCCT) reference standards to assure global comparability.

Data entry and statistical analysis

The collected data were entered into Microsoft Excel for initial sorting and then subjected to statistical analysis using MedCalc software (version can be specified if available). The mean and standard deviation (SD) of HbA1c levels were calculated for each of the four groups. To determine whether observed differences in HbA1c levels between groups were statistically significant, Analysis of Variance (ANOVA) was applied. If ANOVA indicated significance, post hoc tests such as Tukey's HSD were used to identify specific intergroup differences. A p-value < 0.05 was considered statistically significant.

Ethical considerations

Before the commencement of the study, ethical approval was obtained from the Institutional Ethics Committee of RIMS, Ranchi. All participants were thoroughly informed about the study objectives, their role, potential risks, and the voluntary nature of participation. Informed consent was taken in writing from each subject following the Declaration of Helsinki guidelines on human subject research.

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Results

Participant flow

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Figure 1: Showing the participant flow

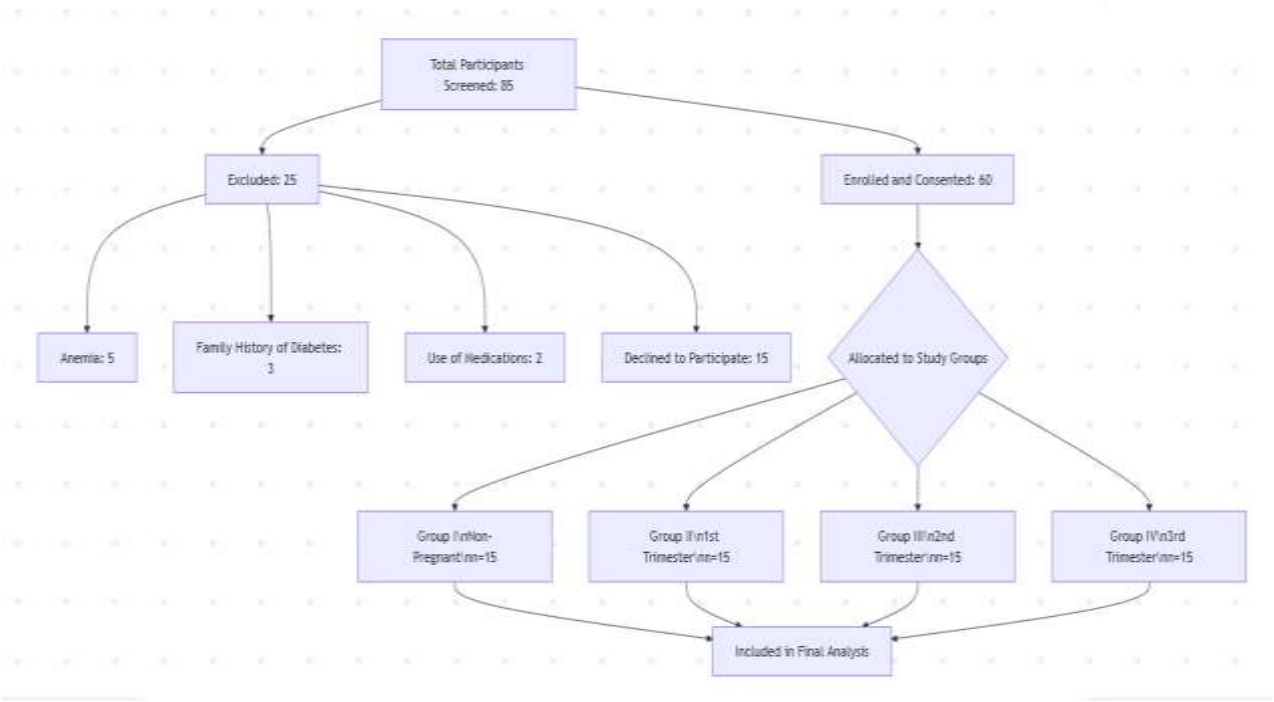

Table 1: Mean HbA1c (%) in non-pregnant and pregnant women across trimesters

Group	Age (Years)	Mean HbA1c (%)	SD
Non-Pregnant	24.2 ± 3.4	5.55	0.30
1st Trimester	24.6 ± 3.2	5.12	0.32
2nd Trimester	24.1 ± 3.6	5.33	0.32
3rd Trimester	24.2 ± 3.3	5.03	0.28

A significant reduction in HbA1c was observed in the 1st trimester compared to the non-pregnant group ($p=0.0007$), while the increase in the 2nd trimester was not statistically significant. In the 3rd trimester, the HbA1c level dropped further, and this decrease was highly significant ($p<0.0001$).

Table 2: P-values and statistical significance between groups

Comparison	p-value	Significance
NP vs 1st Trimester	0.0007	Significant (S)
NP vs 2nd Trimester	0.0622	Not Significant (NS)
NP vs 3rd Trimester	<0.0001	Highly Significant (HS)
1st vs 2nd Trimester	0.0831	Not Significant (NS)
2nd vs 3rd Trimester	0.0108	Significant (S)
1st vs 3rd Trimester	0.4913	Not Significant (NS)

The difference between non-pregnant women and the first trimester is significant ($p = 0.0007$), while the comparison with the third trimester is highly significant ($p < 0.0001$), indicating marked reductions in HbA1c during these stages of pregnancy. However, the difference between non-pregnant women and the second trimester is not

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significant (p = 0.0622), suggesting some overlap in values during this period. Among trimesters, a significant difference is observed between the second and third trimesters (p = 0.0108), indicating a rise in HbA1c later in pregnancy. Other inter-trimester comparisons did not yield significant differences, suggesting relative stability across early and mid-pregnancy.

Discussion

Overview of findings

This study compared the glycated haemoglobin (HbA1c) levels of pregnant women throughout a normal pregnancy to those of non-pregnant women of the same age. The results showed that HbA1c decreased dramatically in the first and third trimesters and increased somewhat in the second. All three trimesters revealed lower HbA1c levels than the non-pregnant control group; however, the third trimester was most noticeable. The average HbA1c value for non-pregnant women was 5.55% ± 0.30. First trimester: 5.12% ± 0.32; second trimester: 5.33% ± 0.32; third trimester: 5.03% ± 0.28. These data suggest that hormonal and metabolic changes during pregnancy can affect glycaemic markers. Although trimester-sensitive, HbA1c can help monitor the mother's glycaemic management.

Comparison with existing literature

Other prominent studies have identified a similar pattern to ours. Nielsen et al. attributed reduced HbA1c levels in early and late pregnancy to spontaneous hemodilution and increased red blood cell turnover [13]. Our study demonstrated significantly lower HbA1c values in the first and third trimesters. O'Kane et al. also identified an early decline, mid-pregnancy surge, and late decline, with trimester-specific values of 5.1%, 4.9%, and 5.0% for the first, second, and third trimesters. Mosca et al. created reference values for several gestational periods and confirmed a significant drop in HbA1c during late pregnancy, which agrees with our data [14]. However, Versantvoort et al. found that late-pregnancy HbA1c levels may rise. This may be due to liver glucose output and placental hormone-induced insulin resistance [15]. These discrepancies emphasise the need for population-specific HbA1c interpretation reference intervals and standards in pregnancy.

Physiological mechanisms behind HbA1c variations

Human haemoglobin A1c levels fluctuate during pregnancy due to several physiological events. Early pregnancy lowers glucose intake and increases transient hypoglycemia due to decreased appetite, illness, and vomiting (hyperemesis gravidarum), which lowers haemoglobin glycation. An increase in erythropoiesis lowers HbA1c because red blood cells live less. This reduces glucose-haemoglobin binding time. Therefore, pregnant women had a considerably smaller first-trimester drop than non-pregnant women (p = 0.0007). In the second trimester, mothers' hunger and food intake improve. Meanwhile, the placenta produces more cortisol, hPL, and progesterone, which increase insulin resistance. This alteration explains the smaller but statistically insignificant second-trimester HbA1c rise (p = 0.0622 compared to non-pregnant). Insulin resistance peaks in the third trimester. Hyperglycemia lowers HbA1c, but insulin synthesis and delayed stomach emptying compensate. These adjustments appear to stabilise glucose levels in healthy pregnancies, with third-trimester readings significantly lower (p < 0.0001 compared to non-pregnant).

Generalizability of the study

The findings of this study provide important insights into trimester-specific variations in HbA1c levels among healthy pregnant women. However, the generalizability of the results may be limited by the relatively small sample size and the single-center design. Participants were primarily from an urban, hospital-based population within a specific geographic region (Ranchi, Jharkhand), which may not reflect the broader demographic and socioeconomic diversity of the general population. Additionally, the exclusion of individuals with comorbidities such as anemia or known diabetes limits applicability to high-risk pregnancies. Therefore, while the trends observed are consistent with other literature, caution should be exercised in extrapolating these results to different populations. Larger, multicenter, and ethnically diverse studies are warranted to enhance external validity.

Conclusion

This study underlines the necessity of realising that non-diabetic pregnant women's HbA1c levels may vary dramatically across trimesters. HbA1c values drop dramatically in the first trimester, rise somewhat in the second, then reduce even more in the third trimester

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compared to non-pregnant women. These oscillations are caused by pregnancy-specific physiological and hormonal alterations, including hemodilution, altered glucose metabolism, increased red blood cell turnover, and placental hormone-induced insulin resistance. The results emphasise the need to interpret HbA1c levels in the context of pregnancy physiology rather than non-pregnant reference periods. HbA1c, a single, non-fasting blood test that is unaffected by short-term glucose fluctuations, may be beneficial for glycaemic surveillance during pregnancy when oral glucose tolerance testing (OGTT) is not practicable or well-tolerated. It should be used with trimester-specific reference values to ensure clinical accuracy. More research with larger and more diverse populations is needed to standardise reference ranges across ethnicities and therapeutic settings. This study supports HbA1c for pregnant glycaemic monitoring; however, more research is needed. When evaluated within the physiological context of pregnancy, HbA1c can contribute to maternal health monitoring as a non-invasive, reliable, and patient-friendly alternative for detecting and managing potential glycaemic abnormalities.

Limitations of the Study

This study has several limitations. First, it employed a cross-sectional design, collecting data at a single point in time from each participant, which restricts the ability to assess longitudinal intra-individual changes across pregnancy. Second, the sample size was relatively small, with only 15 participants in each group, which may limit the statistical power and generalizability of the findings. Third, the study was conducted at a single tertiary care center and included participants from a relatively homogenous urban population, which may not represent the broader population. Lastly, potential confounding factors such as iron status, dietary habits, and subclinical anemia were not controlled or assessed in detail, and these could influence HbA1c levels.

Recommendation

Based on the findings of this study, it is recommended that trimester-specific reference ranges for HbA1c be considered in routine prenatal care. While HbA1c alone may not replace the oral glucose tolerance test (OGTT) for gestational diabetes screening, it can serve as a useful adjunct, particularly in situations where OGTT is impractical or contraindicated. Clinicians should interpret HbA1c values during pregnancy with awareness of physiological changes specific to each trimester, ensuring appropriate clinical decision-making.

Clinical implications and use of HbA1c in pregnancy

This study is significant for using HbA1c to diagnose and monitor pregnancy. Despite its accuracy in diagnosing gestational diabetes mellitus (GDM), pregnant women often struggle with the oral glucose tolerance test (OGTT) due to its fasting restrictions and gastrointestinal discomfort. Haemoglobin A1c (HbA1c) is a good alternative that takes only one non-fasting blood sample and is insensitive to glucose metabolism changes. However, this study shows that pregnancy-specific physiological changes affect HbA1c levels; hence, reference values should be defined for each trimester. These requirements could make HbA1c a good GDM screening tool, especially for women who cannot or should not undertake OGTT. Monitoring HbA1c often throughout pregnancy can help identify high-risk pregnancies or borderline glucose readings, which can lead to macrosomia, pre-eclampsia, or infant hypoglycemia.

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Lists of abbreviations

OGTT- oral glucose tolerance tests
GDM- Gestational diabetes mellitus.
HPLC- High-Performance Liquid Chromatography.
HbA1c- Glycated hemoglobin.
NGSP- National Glycohemoglobin Standardisation Program.
DCCT- Diabetes Control and Complications Trial.

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Conflict of interest

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Author Biography

Dr. Archana is working as a Tutor in the Department of Physiology at RIMS, Ranchi, Jharkhand.
Dr. Anil Kumar Kamal is working as an Associate Professor at the Department of General Surgery at RIMS, Ranchi, Jharkhand.
Dr. Ranjit Kumar Arora is working as a Professor at the Department of Physiology at Palamu Medical College, Daltenganj.
Dr. Priyanka Shrivastava is working as an Associate Professor at the Department of Anaesthesiology at RIMS Ranchi, Jharkhand.
Dr. Deepak Kumar is working as a junior Resident at the Department of General Surgery at RIMS, Ranchi, Jharkhand.
Dr. Ranjit Kumar Rajak is working as a junior Resident at the Department of General Surgery at RIMS, Ranchi, Jharkhand.
Dr. Madhu Kumari is working as a senior Resident at the Department of Gynaecology at RIMS, Ranchi, Jharkhand.

Author Contribution

Dr Archana – Data collection, drafting and interpretation and finalizing and final editing of this manuscript.

Dr Anil Kumar Kamal– Drafting supervision and proofreading of this manuscript.

Dr Ranjit KumarArora/ Dr Priyanka Shrivastava/Dr Madhu Kumari/Dr Deepak Kumar/Dr Ranjit Kumar Rajak -Finalize, conceptualized, briefings, corrections, and final editing of this manuscript.

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