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ORIGINAL ARTICLE

Determining how the gestational changes, including low hemoglobin influence the hba1c and estimated average glucose relationship

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ABSTRACT

Background: Gestational Diabetes Mellitus (GDM) affects up to 14% of pregnancies globally, with accurate glycemic control critical for preventing adverse outcomes. While HbA1c is a standard marker for long-term glucose control, its reliability in pregnant women with anemia is debated. This study explores the association between anemia and glycemic indicators (Glycosylated hemoglobin HbA1c, Random plasma glucose (rPG), and Estimated average glucose (eAG) in pregnant women with GDM. Additionally, it examined the correlation between average glucose levels or eAG, measured by random plasma glucose rPG, and HbA1C.

Methods: A prospective case-control study was carried out over eight months at the Departments of Chemical Pathology and Obstetrics and Gynecology, Dow University of Health Sciences (DUHS). Pregnant women in their second trimester after being diagnosed for GDM through oral glucose tolerance test were classified into anemic (hemoglobin < 10.5 g/dL) and non-anemic groups. Blood samples were collected and analyzed for HbA1c, hemoglobin, and random plasma glucose levels. Data were processed using SPSS (version-26) different variables compared by using independent t-test and correlation analyses at a significance level of $p < 0.05$.

Results: Mean values for eAG, HbA1C, and rPG were 174.22 ± 24.489 mg/dl, 8.342 ± 1.6152%, and 166.21 ± 21.478 mg/dl, respectively. Anemia was associated with significantly higher HbA1C ($p < 0.001$), eAG ($p < 0.001$), and rPG ($p = 0.004$). A strong positive correlation was observed between eAG and rPG ($r = 0.958$, $p < 0.01$), while eAG showed a moderate correlation with HbA1C ($r = 0.501$, $p < 0.01$). In contrast, the correlation between HbA1C and rPG was weaker ($r = 0.331$, $p < 0.01$).

Conclusion: eAG proved to be a good indicator for assessing glycemic control in pregnant non-anemic women with GDM. Anemia significantly affected glycemic metrics, leading to higher HbA1C and glucose levels.

Keywords: Gestational diabetes mellitus, estimated average glucose (eAG), HbA1C, random plasma glucose (rPG), anemia.

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Introduction

Gestational diabetes mellitus (GDM) is a significant global health concern affecting pregnant women, particularly between 24 and 28 weeks of gestation (1). Prevalence of GDM varies widely across different populations, ranging from 1% to 28% (2). According to the International Diabetes Federation (IDF), GDM affects about 14.0% of pregnancies globally, which equals around 20 million births annually. The implications of GDM extend beyond pregnancy, as affected mothers are at an increased risk of developing

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gestational hypertension and pre-eclampsia and may require Cesarean sections for delivery.

Moreover, gestational diabetes mellitus (GDM) increases the risk of long-term health issues such as cardiovascular disease, obesity, and impaired carbohydrate metabolism. This can predispose both the mother and the child to type 2 diabetes mellitus (T2DM) later in life (1, 3).

During pregnancy, physiological changes significantly affect parameters such as hemoglobin and blood glucose levels. Hemoglobin A1C (HbA1C) and average glucose levels have been extensively studied, with recent advancements including continuous glucose monitoring (CGM) for a more accurate representation (4, 5).

Accurately assessing glucose control is crucial for pregnant women with diabetes, as tight glucose control positively affects maternal and fetal health outcomes.. HbA1c is widely recognized as a key marker for long-term glycemic control, reflecting the average plasma glucose level over the lifespan of red blood cells (about 120 days) (6). Despite its utility in general diabetes management, HbA1c's effectiveness in monitoring GDM is debated (7-10). It is influenced by various factors and may not accurately reflect glucose control during pregnancy. HbA1c is formed through the non-enzymatic binding of glucose to hemoglobin, and its concentration depends on both plasma glucose levels and red blood cell lifespan. Conditions such as hemoglobinopathies can affect HbA1c measurements, highlighting the need for cautious interpretation of HbA1c levels in pregnant patients (11). Despite its widespread use in clinical practice, these factors contribute to the uncertainty of HbA1C's role in blood glucose assessment during pregnancy.

Anemia, defined by hemoglobin levels < 10.5 g/dL during the second trimester, is another common condition complicating pregnancy. Anemia could affect the interpretation of HbA1C, a marker for long-term glycemic control (12). HbA1C reflects average blood glucose levels over the previous two to three months but could be compromised by changes in red blood cell turnover during pregnancy (12, 13). The Estimated Average Glucose (eAG), calculated from HbA1C levels, provides an intuitive metric for assessing glycemic control. However, the validity of eAG in pregnant populations, especially those with anemia, requires further investigation (14).

Understanding the influence of low hemoglobin on the HbA1C and eAG relationship is crucial for healthcare providers managing glycemic control in pregnant individuals. Accurate blood glucose level assessment is vital for managing conditions such as GDM. This study

investigated the association between anemia and glycemic control indicators (HbA1C, Random Plasma Glucose rPG and eAG) in diabetic pregnant women. Furthermore, this study also finds the correlation between rPG, eAG and HbA1C.

Methods

This case-control study (Cases were anemic gestational diabetic women, and control subjects were non-anemic gestational diabetic women) was after approval from the Institutional Review Board committee (IRB Ref no IRB- 3430 /DUHS/Approval/2024/122). The study was conducted over six months (from 1-3-2024 to 31-8-2024) at the Department of Chemical Pathology in collaboration with the Department of Gynecology and Obstetrics at Dow University of Health Sciences (DUHS).

The study included pregnant women in their second trimester. Women with a history of pre-pregnancy diabetes, hemoglobinopathies, or chronic illnesses such as liver or renal disease, hypertension, or autoimmune diseases were not included in the study. The participants were recruited through purposive sampling from the gynecology and obstetrics outpatient department (OPD). WHO sample size calculator is used to find the sample size. To obtain reliable results, it's essential to consider a confidence level of 95% and a margin of error. Our results include a 5% margin of error and a sample size 112. The study comprised two groups: Non-anemic and anemic pregnant women diagnosed with GDM via the Oral Glucose Tolerance Test (OGTT).

Based on the American Diabetic Association criteria, which include fasting blood glucose levels between 92 and 125 mg/dl, a 1-hour post-glucose load of >180 mg/dl, and a 2-hour post glucose load between 153 and 199 mg/dl (15,16). Anemia was defined as hemoglobin levels < 10.5 g/dL during the 2nd trimester of pregnancy (12).

Upon obtaining informed written consent, 5 ml of blood was withdrawn from each patient. Blood samples were analyzed for HbA1c, hemoglobin, and random plasma glucose (rPG) levels. The eAG was calculated using the A1C-derived average glucose (ADAG) equation (17). Sociodemographic information, past medical history, and medication history were collected using a predefined proforma.

Data entry and analysis were conducted using SPSS version 22.0. For continuous variables, means and standard deviations (SD) were calculated, while categorical variables were summarized using frequencies and percentages. To compare eAG and HbA1c levels between the anemic and non-anemic groups, we used the independent sample t-test. We assessed the relationships between variables using Pearson correlation coefficients. P values <0.05 were considered significant.

Results

The study included 112 women with gestational diabetes. Among them, 67 (59.8%) were anemic while 45 (40.2%) were non anemic. The distributions of different variables among anemic and non-anemic patients are shown in table 1.

Iron supplementation greatly impacts the status of anemia. Iron supplementation decreases the frequency of anemia. There were statistically significant differences observed between anemic and non-anemic GDM patients on iron supplementations with p-value 0.04 as shown in figure 1.

Table 1: Demographics of Women with gestational diabetes

Characteristics	Anemia	
	Present N(%)	Absent N(%)
Age		
18-25 years	31 (46.3)	17 (37.8)
26-35 years	36 (53.7)	28 (62.2)
Previous Pregnancy		
Yes	44 (65.7)	23 (51.1)
No	23 (34.3)	22 (48.9)
Past GDM		
Yes	12 (17.9)	9 (20.0)
No	55 (82.1)	36 (80.0)
Complications in Pregnancy		
Yes	27 (40.3)	25 (55.6)
No	40 (59.7)	20 (44.4)
Iron Supplementation		
Yes	31 (46.3)	13 (28.9)
No	36 (53.7)	31 (71.1)
Glucose Monitoring Frequency		
Daily	8 (11.9)	11 (24.4)
Weekly	34 (50.7)	20 (44.4)
Occasionally	15 (22.4)	10 (22.2)
Rarely	10 (14.9)	4 (8.9)
Oral hypoglycemic Medication		
Yes	43 (64.2)	32 (71.1)
No	24 (35.)	13 (28.9)

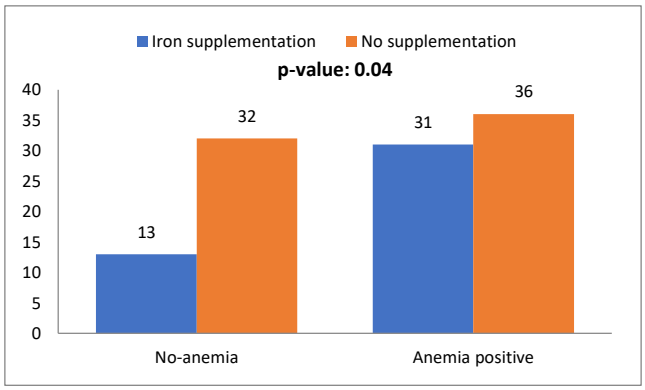

Figure 1: Iron supplementation between anemic and non-anemic patients

The independent sample t test was applied to find the possible differences among different variables in anemic and non-anemic GDM patients. The analysis revealed that the mean HbA1C was significantly higher in the anemic group (mean±SD = 9.397±1.01) compared to the non-anemic group (mean±SD = 6.771±0.90), the difference is highly statistically significant with p-value <0.001 and 95% CI -32.95-16.74. Similarly, the eAG and rPG were significantly higher among anemic group as compared to non-anemic group with p-values <0.001 and 0.008 respectively. The details of each parameter are summarized in table 2.

Table 2: Glycemic Control Indicators in Diabetic Pregnant Women

	Anemia	N	Mean ± SD	P-value	95% CI
HbA1C	No	45	6.771±0.90	<0.001	-32.95-16.74
	Yes	67	9.397±1.01		
eAG (mg/dl)	No	45	159.38±26.6	<0.001	-33.74-15.89
	Yes	67	184.19±16.8		
rPG (mg/dl)	No	45	159.11±26.1	0.008	-19.80-3.9
	Yes	67	170.99±16.2		

The strongest correlation was observed between eAG and rPG (r = 0.958), followed by eAG and HbA1C (r = 0.501), and lastly, HbA1C and rPG (r = 0.331) (Table 3). These findings indicate that eAG is the most reliable indicator of glycemic control in this study population, making it a superior measure for assessing blood glucose levels in pregnant women with GDM compared to HbA1C.

Table 3: Pearson Correlation Coefficients between Glycemic Control Indicators in GDM Patients

Glycemic Indicator	eAG	HbA1C	rPG
eAG	1	0.501** (p=0.000***)	0.958** (p=0.000***)
HbA1C	0.501** (p=0.000***)	1	0.331** (p=0.000***)
rPG	0.958** (p=0.000***)	0.331** (p=0.000***)	1

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

Discussion

Effective glycemic control in pregnant women with GDM is crucial to minimize adverse outcomes. Traditionally, HbA1C has served as the gold standard for assessing long-term glycemic control. Emerging evidence suggests that alternative indicators like eAG may offer additional advantages, particularly in reflecting real-time glucose levels. This study sought to examine the impact of anemia on various glycemic control indicators—HbA1C, rPG, and eAG—while exploring the relationships between these measures.

Our study found that the mean eAG was 174.22 ± 24.489 mg/dl, the mean HbA1C was 8.342 ± 1.6152%, and the mean rPG was 166.21 ± 21.478 mg/dl. These values are consistent with those reported in similar studies of diabetic pregnant women (9, 15), indicating that our sample's glucose levels were elevated, as expected in GDM cases. The high mean eAG and rPG levels emphasize the importance of effective glycemic management in this population.

A significant finding from our study was the impact of anemia on glycemic control indicators. Anemic participants exhibited significantly higher mean HbA1C (9.397) compared to non-anemic participants (6.771), with a mean difference of -2.626 ($p < 0.001$). Also, eAG and rPG levels were markedly elevated in the anemic group. These findings suggest that anemia exacerbates hyperglycemia, likely due to altered red blood cell turnover affecting HbA1C readings and potentially influencing glucose metabolism. Our results align with existing literature, which suggests that anemia can distort HbA1C measurements, resulting in

higher observed values in anemic individuals (16-18).

The study observed a robust positive correlation between eAG and rPG ($r = 0.958$, $p < 0.01$), indicating a high degree of alignment between these two measures of glucose levels. This finding supports the notion that eAG is an effective real-time indicator of blood glucose levels and correlates closely with random glucose measurements. Previous studies reported similar results (9), which also found a strong correlation between eAG and rPG, reinforcing eAG's reliability in reflecting current glucose levels.

The moderate positive correlation between eAG and HbA1C ($r = 0.501$, $p < 0.01$) suggests a moderate association between these measures. While eAG indicates current glucose levels, HbA1C offers additional insights into long-term glucose control. This moderate correlation is consistent with other research suggesting that eAG and HbA1C measure different aspects of glucose control (9).

Conversely, the weaker positive correlation between HbA1C and rPG ($r = 0.331$, $p < 0.01$) indicates that HbA1C is less reflective of real-time glucose levels compared to eAG. This finding aligns with research suggesting that HbA1C, while helpful in assessing long-term glucose trends, may not capture fluctuations in blood glucose levels as effectively as eAG (19).

The significant association between anemia and elevated HbA1C, eAG, and rPG levels in our study underscores the impact of anemia on glycemic control indicators. Specifically, the mean HbA1C was significantly higher in anemic participants, and eAG and rPG levels were alsoelevated. These results are consistent with the findings of Hashimoto *et al.* (20), who reported elevated HbA1C levels in late pregnancy attributed to increased iron deficiency. This suggests that anemia may confound glycemic control measurements, leading to falsely high HbA1C values.

Our findings corroborate those of Coban *et al*. (21), who observed that iron deficiency could result in falsely high HbA1C levels. However, our findings contrast with those of Firat*et al*. (22), who noted a decrease in HbA1C levels during the first half of pregnancy. The discrepancy may be attributed to variations in the stages of pregnancy or differences in anemia severity across studies.

Overall, this study emphasizes the significant impact of anemia on glycemic control indicators and endorses using eAG as a more reliable measure of real-time glucose levels compared to HbA1C. These findings have implications for managing diabetes in pregnant women, emphasizing the need to consider anemia when evaluating glycemic control.

Conclusion

In conclusion, eAG emerges as the most reliable indicator of glycemic control among the three measures assessed in this study, particularly for real-time glucose monitoring in non-anemic gestational diabetic women. The impact of anemia on HbA1C and glucose levels highlights the importance of considering anemia status when evaluating glycemic control. These findings support using eAG for more accurate glycemic monitoring in non-anemic pregnant women with GDM.

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References

1. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nat Rev Dis Primers. 2019 Jul 11; 5(1):47. Doi: 10.1038/s41572-019-0098-8.
2. Dewi RS, Isfandiari MA, Martini S, Yi-Li C. Prevalence and risk factors of gestational diabetes mellitus in Asia: a review. Journal of Public Health in Africa. 2023; 14(s2):7.
3. Modzelewski R, Stefanowicz-Rutkowska MM, Matuszewski W, Bandurska-Stankiewicz EM. Gestational diabetes mellitus—recent literatures review. J Clin Med. 2022 Sep 28; 11(19):5736. Doi: 10.3390/jcm11195736.
4. Galindo RJ, Aleppo G. Continuous glucose monitoring: The achievement of 100 years of innovation in diabetes technology. Diabetes Res Clin Pract. 2020 Dec; 170:108502. Doi: 10.1016/j.diabres.2020.108502.
5. Cappon G, Vettoretti M, Sparacino G, Facchinetti A. Continuous Glucose Monitoring Sensors for Diabetes Management: A Review of Technologies and Applications. Diabetes Metab J. 2019 Aug; 43(4):383-97. Doi: 10.4093/dmj.2019.0121.
6. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF diabetes atlas: estimation of global and regional gestational diabetes mellitus prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. Diabetes Res Clin Pract. 2022 Jan; 183:109050.Doi:10.1016/j.diabres.2021.109050.
7. Yin B, Hu L, Meng X, Wu K, Zhang L, Zhu Y, et al. Association of higher HbA1c within the normal range with adverse pregnancy outcomes: a cross-sectional study. Acta Diabetol. 2021 Aug; 58(8):1081-89. Doi: 10.1007/s00592-021-01691-0.
8. Gyasi-Antwi P, Walker L, Moody C, Okyere S, Salt K, Anang L, et al. Global prevalence of gestational diabetes mellitus: a systematic review and meta-analysis. New American Journal of Medicine. 2020; 1(3):1-10.
9. Law GR, Gilthorpe MS, Secher AL, Temple R, Bilous R, Mathiesen ER, et al. Translating HbA1c measurements into estimated average glucose values in pregnant women with diabetes. Diabetologia. 2017; 60(4):618-24.
10. Bozkurt L, Göbl CS, Leitner K, Pacini G, Kautzky-Willer A. HbA1c during early pregnancy reflects beta-cell dysfunction in women developing GDM. BMJ Open Diabetes Res Care. 2020 Nov; 8(2):e001751. Doi: 10.1136/bmjdrc-2020-001751.
11. Hong JGS, Fadzleeyanna MYN, Omar SZ, Tan PC. HbA1c at term delivery and adverse pregnancy outcome. BMC Pregnancy Childbirth.2022; 22(1):679.
12. Noshiro K, Umazume T, Hattori R, Kataoka S, Yamada T, Watari H. Hemoglobin concentration during early pregnancy as an accurate predictor of anemia during late pregnancy. Nutrients. 2022 Feb 17; 14(4):839. Doi: 10.3390/nu14040839.
13. Klonoff DC. Hemoglobinopathies and hemoglobin A1C in diabetes mellitus. SAGE Publications Sage CA: Los Angeles, CA; 2020. p. 3-7.
14. Sriwimol W, Choosongsang P, Choosongsang P, Petkliang W, Treerut P. Associations between HbA1c-derived estimated average glucose and fasting plasma glucose in patients with normal and abnormal hemoglobin patterns.

Scand J Clin Lab Invest. 2022 May; 82(3):192-8. Doi: 10.1080/00365513.2022.2040051.
15. Sisay M, Edessa D, Ali T, Mekuria AN, Gebrie A. The relationship between advanced glycation end products and gestational diabetes: A systematic review and meta-analysis. PLoS One. 2020 Oct 21; 15(10):e0240382.
16. Renz PB, Hernandez MK, Camargo JL. Effect of iron supplementation on HbA1c levels in pregnant women with and without anaemia. Clin Chim Acta. 2018 Mar; 478:57-61. Doi: 10.1016/j.cca.2017.12.028.
17. Kim HY, Kim J, Noh E, Ahn KH, Cho GJ, Hong SC, Oh MJ, Kim HJ. Pre pregnancy hemoglobin levels and gestational diabetes mellitus in pregnancy. Diabetes. Res. Clin. Pract. 2021 Jan; 171:108608. Doi: 10.1016/j.diabres.2020.108608.
18. Firat A, Katlan DC, Uzunay N. Impact of Iron Deficiency Anemia on Hemoglobin A1C Levels in Diabetic and Non-Diabetic Pregnant Women. Clin Exp Obstet Gynecol. 2024; 51(1):24.
19. Agbozo F, Abubakari A, Narh C, Jahn A. Accuracy of glycosuria, random blood glucose and risk factors as selective screening tools for gestational diabetes mellitus in comparison with universal diagnosing. BMJ Open Diab Res Care. 2018; 6(1):e000493.
20. Hashimoto K, Koga M. Influence of iron deficiency on HbA1c levels in pregnant women: comparison with non-pregnant women. J. Clin. Med. 2018, 7(2), 34; https://doi.org/10.3390/jcm7020034
21. Coban E,Ozdogan M,Timuragaoglu A. Effect of iron deficiency anemia on levels of hemoglobin A1c in nondiabetic patients. Acta haematologica. 2004; 112(3):126-8.
22. Firat A, Katlan DC, Uzunay N. Impact of Iron Deficiency Anemia on Hemoglobin A1c Levels in Diabetic and Non-Diabetic Pregnant Women. Clin. Exp. Obstet. Gynecol. 2024 jan 22; 51(1):24.

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