Association between mid-pregnancy HbA1c values and anemia in women without gestational diabetes

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Research Article

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Abstract

**Background:** Previous studies have examined the association between glycated hemoglobin (HbA1c) and hemoglobin (Hb) levels among non-diabetic people. However, there are no reports relating to the correlation between HbA1c values and anemia in women without gestational diabetes. We, therefore, examined whether there is an association between mid-pregnancy HbA1c values and anemia in women without gestational diabetes.

**Methods:** This retrospective cohort study was conducted at the Affiliated Hospital of Jining Medical University, Jining, China, from January to December 2019. Cases of single pregnancies in women above 18 years old, with recorded HbA1c values at 14-27^{+6} weeks of gestation, were examined. Women with pregestational diabetes mellitus (GDM) or the development of GDM were excluded. The association between variations in mid-pregnancy HbA1c values and anemia was examined.

**Results:** Among 8553 women without gestational diabetes, univariate analysis results demonstrated a significant positive relationship between \( P<.001 \) mid-pregnancy HbA1c values and anemia, after adjusting for confounding factors. Multivariate piecewise linear regression showed that anemia decreased when HbA1c values were <4.6% (OR 0.40; 95% CI, 0.15-1.04; \( P=.592 \)) and increased when HbA1c values were was >4.6 % (OR 3.83; 95% CI, 2.95-4.98; \( P<.0001 \)).

**Conclusion:** This study showed a nonlinear relationship between mid-pregnancy HbA1c values and anemia in women without gestational diabetes. When the HbA1c values increased to the inflection point, they were positively correlated with anemia. Further studies are needed to explore this relationship and the mechanisms involved in the future.

Background

According to the WHO guidelines, anemia in pregnancy is defined by the hemoglobin concentration (Hb) < 110g/L. Anemia in pregnant women is a public health problem, especially in developing countries [1]. Stevens et al [2] reported that the global prevalence of anemia in pregnancy is 38%. The prevalence of anemia in a total of 467057 prenatal women who participated in a perinatal health-care surveillance system, and delivered babies from 1993 to 2005, was found to be 39.6% [3]. A large multicenter retrospective study (n = 44,002) showed that anemia was 23.5% in pregnant Chinese women [4]. Anemia is one of the most prevalent complications during pregnancy and is generally considered to be a risk factor for adverse pregnancy outcomes. Anemia in pregnancy may threaten the life of both mother and fetus. Maternal anemia is a further risk factor for hypertension, miscarriage, primary cesarean delivery, diabetes, placental abruption, chorioamnionitis, and postpartum hemorrhage [5–8]; and fetuses are more likely to be diagnosed with fetal distress, preterm birth, and low birth weight [9–11].

Glycated hemoglobin (HbA1c) is a substance formed by the reaction between the amino (N) terminal of hemoglobin and hexose, reflecting time-averaged blood glucose during the previous two to three months. The HbA1c value is essential for the diagnosis and management of diabetes, and is the best predictor of
disease complications. The consensus panel of the International Association of Diabetes and Pregnancy Study Groups recommended that a HbA1c value of $\geq 6.5\%$ be used for the diagnosis of gestational diabetes mellitus (GDM), with a HbA1c value $\geq 6.0\%$ indicating a high risk of GDM \cite{12, 13}. Higher pregnancy HbA1c levels are significantly associated with increased risks of gestational hypertension or pre-eclampsia, placental abruption, cesarean section \cite{14–16}, and adverse birth outcomes (preterm birth, macrosomia, and LGA) \cite{17–19}.

Most previous studies have reported that the HbA1c value is positively associated with anemia in different populations \cite{20, 21}. Nondiabetic individuals with anemia have presented with significantly higher HbA1c measurements than those obtained from non-anemic subjects \cite{22}. Anemia is one of the diseases that most often affects HbA1c values, and many studies have investigated their relationship. However the mechanism for this remains unclear \cite{23, 24}. A meta-analytic study showed that the relationship between HbA1c and anemia remains inconclusive and that further studies are required for clarification \cite{25}. Research has, furthermore, revealed that anemia should be corrected in the diagnostic threshold, well before the HbA1c value indicates diabetes \cite{26}.

However, there are no scientific dissertations about the correlation between mid-pregnancy HbA1c values and anemia. HbA1c values and anemia are known to be related to adverse pregnancy outcomes. Exploring the relationship between HbA1c values and anemia in pregnancy may, therefore, provide new insights into its occurrence and development mechanism. Hence, this study evaluated the relationship between mid-pregnancy HbA1c values with anemia.

**Methods**

**Study Design and Population**

This single-center observational study was performed at the Affiliated Hospital of Jining Medical University in China, from January to December 2019, based on electronic records. Cases of single pregnancies in women above 18 years old, with recorded HbA1c values during 14–27+6 weeks of gestation were included. Women with pre-GDM or the development of GDM were excluded. The data were anonymous, and the requirement for patient informed consent was therefore waived. Patient data confidentiality was kept and was in compliance with the Declaration of Helsinki. This study was approved by the Human Ethics Committee of the Affiliated Hospital of Jining Medical University.

The analyzed data were extracted from the medical record information system of the Affiliated Hospital of Jining Medical University. Data on 16,703 pregnant women who gave live birth in the hospital were continuously collected. The following maternal information was included: age, height, pre-pregnancy weight, and pre-pregnancy body mass index (BMI), calculated in kg/m$^2$. Diabetes and hypertension histories were recorded. Obstetric history was assessed in terms of the time of detecting HbA1c and HbA1c values, gestational age of delivery, pregnancy times, parity, pre-eclampsia, anemia, chronic hypertension with pregnancy and hypothyroidism during pregnancy.
Blood was drawn in the morning after a fasting period of at least 8 hours. The HbA1c value in the blood was measured using an ARKRAY HA-8180 automatic glycosylated hemoglobin analyzer (HA-8180) based on the principle of ion-exchange, high-performance, liquid chromatography.

**Statistical Analysis**

General information and the obstetric history of the subjects were first described (Table 1). The mean and standard deviation was used for continuous variables in a normal distribution, and the median and quartile was used for continuous variables in a non-normal distribution. Categorical variables were expressed in terms of frequencies or percentages. A univariate analysis (Table 2) was used to examine whether mid-pregnancy HbA1c values and other variables correlated with anemia. To maximize statistical power and minimize bias that might occur if missing data were excluded from analyses, we used multivariate multiple imputations to ascribe missing data relating to pre-pregnancy BMI [27]. We examined the relationship between mid-pregnancy HbA1c values and anemia using the smooth curve fitting, after adjustment for potential confounders (Fig. 2). We further used a multivariate piecewise linear regression model to assess independent correlations between mid-pregnancy HbA1c values and anemia, according to the smooth curve fitting (Table 3). Two-sided p values < 0.05 were regarded as significant differences.
Table 1
Baseline characteristics of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values (n = 8533)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>29.34 ± 4.67</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>22.45 ± 3.59</td>
</tr>
<tr>
<td>HbA1c values</td>
<td>4.96 ± 0.29</td>
</tr>
<tr>
<td>Time of detecting HbA1c, gw</td>
<td>25.31 ± 1.14</td>
</tr>
<tr>
<td>Gestational age at delivery, gw</td>
<td>39.08 ± 1.37</td>
</tr>
<tr>
<td>Pregnancy times</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2327 (27.27%)</td>
</tr>
<tr>
<td>2</td>
<td>2620 (30.70%)</td>
</tr>
<tr>
<td>≥ 3</td>
<td>3586 (42.03%)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3496 (40.97%)</td>
</tr>
<tr>
<td>2</td>
<td>4456 (52.22%)</td>
</tr>
<tr>
<td>≥ 3</td>
<td>581 (6.81%)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>203 (2.38%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1176 (13.78%)</td>
</tr>
<tr>
<td>Chronic hypertension with pregnancy</td>
<td>53 (0.62%)</td>
</tr>
<tr>
<td>Hypothyroidism in pregnancy</td>
<td>1669 (19.56%)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or n (%).

Abbreviation: BMI, body mass index; gw, gestational week; HbA1c, glycated hemoglobin A1c.
Table 2
Univariate Analysis of Anemia

<table>
<thead>
<tr>
<th></th>
<th>Statistics</th>
<th>Effect size</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>4.96 ± 0.29</td>
<td>2.28 (1.84, 2.82)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Time of detecting HbA1c, gw</td>
<td>25.31 ± 1.14</td>
<td>1.06 (1.00, 1.11)</td>
<td>0.0531</td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>29.34 ± 4.67</td>
<td>0.98 (0.97, 0.99)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>22.34 ± 3.50</td>
<td>0.94 (0.93, 0.96)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Prepregnancy BMI category, kg/m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18.5</td>
<td>780 (9.14%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>18.5–24.9</td>
<td>5729 (67.14%)</td>
<td>0.79 (0.65, 0.97)</td>
<td>0.0239</td>
</tr>
<tr>
<td>25-29.9</td>
<td>1319 (15.46%)</td>
<td>0.66 (0.51, 0.84)</td>
<td>0.0009</td>
</tr>
<tr>
<td>≥30</td>
<td>307 (3.60%)</td>
<td>0.35 (0.22, 0.57)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Not recorded</td>
<td>398 (4.66%)</td>
<td>0.74 (0.53, 1.04)</td>
<td>0.0869</td>
</tr>
<tr>
<td>Gestational age at delivery, gw</td>
<td>39.08 ± 1.37</td>
<td>1.08 (1.03, 1.13)</td>
<td>0.0022</td>
</tr>
<tr>
<td>Pregnancy times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2327 (27.27%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2620 (30.70%)</td>
<td>1.40 (1.19, 1.66)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>≥3</td>
<td>3586 (42.03%)</td>
<td>1.35 (1.15, 1.58)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3496 (40.97%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4456 (52.22%)</td>
<td>1.52 (1.33, 1.73)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>≥3</td>
<td>581 (6.81%)</td>
<td>1.47 (1.14, 1.88)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>203 (2.38%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8330 (97.62%)</td>
<td>1.66 (1.02, 2.70)</td>
<td>0.0418</td>
</tr>
<tr>
<td>Chronic hypertension with pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Significant results (P < 0.05) are indicated in bold.

Abbreviation: BMI, body mass index; gw, gestational week; HbA1c, glycated hemoglobin A1c.
### Table 3
The Results of Two-Linear Regression Model

<table>
<thead>
<tr>
<th>Inflection point of HbA1c value (%)</th>
<th>Effect size</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4.6</td>
<td>0.40</td>
<td>(0.15, 1.04)</td>
<td>0.0601</td>
</tr>
<tr>
<td>≥ 4.6</td>
<td>3.83</td>
<td>(2.95, 4.98)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>


Adjusted: time of detecting HbA1c, maternal age, prepregnancy BMI, gestational age at delivery, pregnancy times, parity, preeclampsia, chronic hypertension with pregnancy, hypothyroidism in pregnancy.

All the analyses were performed with the statistical software package R (http://www.R-project.org, The R Foundation) and Empower Stats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). *P* values less than 0.05 (two-sided) were considered statistically significant.

## Results

### Baseline Characteristics of Participants

There were 16703 deliveries between January and December 2019. We excluded 2728 pregnancies: 1993 with GDM, 176 with pre-GDM, 70 with a maternal age of < 18 years, 489 with twins or multiple pregnancies, and 5442 with missing HbA1c values during their 14−27+6 weeks of gestation. Finally, the data of 8533 single pregnancies was analyzed (Fig. 1). The clinical characteristics are presented in Table 1. The mean age of the participants was 29.34 ± 4.67 years, the pre-pregnancy mean BMI was 22.45 ± 3.59 kg/m², HbA1c values were 4.96 ± 0.29%, 203 (2.38%) had preeclampsia, 1176 (13.78%) had anemia, 53 (0.62%) had chronic hypertension, and 1669 (19.56%) had hypothyroidism during pregnancy.

### Univariate Analysis of Factors Affecting Anemia
Univariate linear regression analysis was performed to determine the relationships between other variables and anemia. As shown in Table 2, we observed significant positive correlations between anemia and HbA1c values, gestational age at delivery, pregnancy times, parity, preeclampsia and chronic hypertension with pregnancy \((p < 0.05)\). Negative correlations between anemia and both maternal age and pre-pregnancy BMI \((p < 0.05)\) were identified. There were no significant correlations between anemia and the time of detecting HbA1c and hypothyroidism in pregnancy \((p > 0.05)\).

**Non-Linear Relationship Between HbA1c Values and Anemia**

As shown in Fig. 2, the smooth curve fitting showed a nonlinear relationship between HbA1c values and anemia after adjusting for time of detecting HbA1c, maternal age, prepregnancy BMI, gestational age at delivery, pregnancy times, parity, preeclampsia, chronic hypertension with pregnancy and hypothyroidism in pregnancy. There was a two-stage change and a breakpoint in this curve. When the HbA1c was less than the breakpoint, there was no relationship between HbA1c and anemia. When the HbA1c was greater than the breakpoint, there was a positive relationship between HbA1c and anemia. As shown in Table 3, we further analyzed the threshold effect based on the curve fitting, and the data indicated that the inflection point of HbA1c was 4.6%. HbA1c had no relationship with anemia when HbA1c was less than 4.6% \((OR 0.40; 95\% CI, 0.15–1.04; P = .0592)\). Anemia increased as HbA1c increased when HbA1c was greater than 4.6% \((OR 3.83; 95\% CI, 2.95–4.98; P < .0001)\).

**Discussion**

This retrospective study examined the relationship between mid-pregnancy HbA1c values and anemia. We found that a nonlinear relationship existed between mid-pregnancy HbA1c values and anemia. The different correlations of HbA1c values and anemia in pregnancy were found on the left and right sides of the inflection point \((HbA1c values = 4.6\%)\). HbA1c values, as assessed at baseline, were positively associated with anemia in pregnancy on the right side of the inflection point, but the association on the left side of the inflection was not statistically significant.

In this study, we found that by using a two-piecewise linear regression model, the HbA1c value was positively associated with anemia in pregnancy on the right side of the inflection point, which is similar to Lai et al’s finding \([28]\) that Hb levels within the normal range have a negative relationship with HbA1c values when the model is further adjusted for covariates \((\beta = -0.042, 95\% CI: -0.073,-0.012)\). The present study further showed an association between HbA1c values and anemia in pregnancy. However, an interesting finding was that it is a nonlinear relationship, with a cut-off threshold of 4.6%. Kim C et al’s study \([29]\) showed that women with anemia were associated with an increased odds of HA1c \(\geq 5.5\%\) \((odds ratio = 1.39, 95\% CI, 1.11–1.73)\) after adjustment for age, race/ethnicity, and waist circumference, but not with a greater odds of HbA1c \(\geq 6.5\%\) \((odds ratio = 0.79, 95\% CI, 0.33–1.85)\), which might be related to ethnographic differences. Hashimoto K, et al’s study \([30]\) of 47 pregnant Japanese women at 21–36 gestational weeks showed that HA1c levels were raised in relation to iron deficiency. Rajagopal et al.
reported that the HbA1c level increased as the severity of anemia increased in a population of 150 non-diabetic women [31]. A review explained that the variability of results for HbA1c values and anemia indicated that the impact of anemia on HbA1c is multifactorial and multidimensional and that further studies are needed to identify the key factors that affect HbA1c levels [32].

Our study has some strengths. Firstly, we not only used a generalized linear model to evaluate the linear relationship between the HbA1c value and anemia in pregnancy, we also found non-linearity. Secondly, this study is an observational study and susceptible to potential confounding variables. We, therefore, used strict statistical adjustment to minimize residual confounders. Third, we obtained the positive finding that when the HbA1c value is greater than 4.6%, and for every 0.1 unit increase in HbA1c value, anemia in pregnancy increases by 3.83. The clinical value of this finding is that the association of the HbA1c value and anemia in pregnancy can only be observed when the HbA1c value reaches a certain threshold. Furthermore, some potential limitations should be discussed. Firstly, it is a retrospective study that was able only to reveal a nonlinear relationship between HbA1c values and anemia in pregnancy. As such, it is difficult to distinguish between cause and effect, requiring further verification by prospective studies. Secondly, the data collection was monocentric and the study population contained only women without gestational diabetes. The findings of this study cannot, therefore, be used for gestational diabetes mellitus and a non-pregnant population. Third, the diagnosis of anemia in pregnancy was extracted from the admission diagnoses on the hospital information system, and presented as a categorical variable. We did not collect Hb levels and mean corpuscular volume levels, so we were unable to evaluate the relationship between HbA1c levels and Hb levels.

Conclusions

The current article describes important findings that are useful in advancing our understanding of the relationship between anemia in pregnancy and HbA1c level. It was found that HbA1c values (HbA1c > 4.6%) are associated with an increased odds of anemia in pregnancy, and that this relationship is nonlinear.

Abbreviations

HbA1c, glycated hemoglobin; Hb, hemoglobin; BMI, body mass index; GDM, gestational diabetes mellitus.

Declarations

Acknowledgment

The authors thank all the staff members in our institution.

Author Contributions
All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas. They took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

**Funding**

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**Availability of data and materials**

The datasets generated and/or analyzed during the current study are not publicly available due to contracts with research participants but are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The data were anonymous, and the requirement for patient informed consent was therefore waived. Patient data confidentiality was kept and was in compliance with the Declaration of Helsinki. This study was approved by the human ethics committee of the Affiliated Hospital of Jining Medical University (2022-03-C008).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**


Figures
Figure 1

Flowchart of Study Population
Figure 2

Association between HbA1c and anemia in pregnancy. A threshold, nonlinear association between HbA1c and anemia in pregnancy was found in a generalized additive model (GAM). Adjusted for time of detecting HbA1c, maternal age, prepregnancy BMI, gestational age at delivery, pregnancy times, parity, preeclampsia, chronic hypertension with pregnancy, hypothyroidism in pregnancy.