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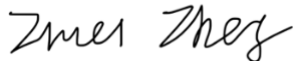

04/10/2023

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Statistical methods for modeling the association between repeated measures of hemoglobin during pregnancy and adverse birth outcomes.

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Statistical methods for modeling the association between repeated measures of hemoglobin during pregnancy and adverse birth outcomes.

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B.Ec., China Pharmaceutical University, 2020

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Abstract

Statistical methods for modeling the association between repeated measures of hemoglobin during pregnancy and adverse birth outcomes

By Ziwei Zhang

Introduction:
Maternal nutrition status during pregnancy plays a crucial role in the health, growth, and development of the fetus and the newborn infant. While some studies may investigate one single measurement, more measurements will provide more information about the biomarker trajectory and how this will associate with birth outcomes.

Methods:
We applied 7 methods that model the association between a longitudinal biomarker and a binary, time-invariant outcome: 1) Window-specific regression; 2) Min/Max/Mean value; 3) Multivariable logistic regression; 4) Conditional Method; 5) Distributed Non-Linear Model; 6) Two-Stage Mixed Effect Model; 7) Generalized Additive Mixed Model (GAMM). We examined the repeated measures of hemoglobin (Hb) concentration across pregnancy in association with low birth weight (LBW), preterm birth (PTB), and small for gestational age (SGA) using the pregnancy woman data from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) which contains 8 datasets from different countries.

Results: 1) The parallel models identified the sensitive window of SGA in 3rd trimester and that of preterm in 1st and 2nd trimesters. 2) Model using maximum Hb detected a risk of higher maximum value Hb in preterm. 3) Multivariable logistic regression indicated that a lower value in 2nd trimester tends to be associated with the probability of SGA. 4) Distributed Non-Linear Model indicated a sensitive window of SGA in 2nd trimester. 5) Two-Stage mixed effect model found that a higher initial value of Hb at the start of gestation significantly decreased the risk of LBW and preterm and a rapid increase in Hb or slower decrease in Hb during pregnancy will decrease the risk of SGA. 6) GAMM shown a significantly lower Hb value in women with preterm around 1st and 2nd trimester.

Conclusions: Methods 4, 5, 6 are preferred when investigators are interested in the biomarker pattern. GAMM emphasizes visualization while others could provide effect estimations. Our study provides extremely useful insight into the pros and cons of statistical methods modeling time-varying predictors in association with not-time-varying outcomes.

Keywords: Biomarkers, Birth outcomes, Repeated measures, Statistical methods

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Introduction

Maternal nutrition status during pregnancy plays a crucial role in the health, growth, and development of the fetus and the newborn infant (Emmett, Jones, & Golding, 2015). For research in pregnant women, it is typical to have repeated measures of biomarkers during pregnancy and a single measurement of birth outcome or child growth. (Cando, Dispo, & Tantengco, 2022; Marvin-Dowle, Burley, & Soltani, 2016; Tous, Villalobos, Iglesias, Fernández-Barrés, & Arija, 2020; Wright et al., 2023). Lots of study examined the Hb value at different time point and applied different methods to investigate the association. But most of them only focus on one single measurement (Liu et al., 2022), average level (Peng et al., 2022), acute value, change in Hb (Jwa, Fujiwara, Yamanobe, Kozuka, & Sago, 2015), multiple logistic regression(Wu et al., 2022) or multiple cross-sectional analysis (Go et al., 2022). These approaches can provide an understanding of the association between biomarker and outcomes to identify potential risk factors. However, there are some assumptions that may not be satisfied in real-world data (Conlin, Hoffman, Steinley, & Sher, 2022) and these approaches don't consider the longitudinal biomarker value. Very few studies provide an overview of statistical approaches to model repeated measurements on time-invariant outcomes in nutrition research, especially with accounting for the effect of time and biomarker trajectory over time.

While some studies focus on a single measurement in association with adverse birth outcome, examining multiple measurements provides more information about the trajectory of the exposure and its association with birth outcomes. To address this research gap, we aim to examine, illustrate, interpret, and compare different methods that could be utilized to investigate the association between longitudinal exposure and outcome. By exploring various methods that model time-varying biomarkers or other exposures in relation to non-time-varying birth outcomes, we seek to shed light on the advantages and disadvantages of each approach. The results of this study could have implications for the field of public health, as it could provide researchers with a better understanding of the most effective ways to investigate the relationship between longitudinal exposures and birth outcomes. Moreover, by applying

these methods to larger sample sizes, this study could yield useful findings that could inform public health interventions aimed at decreasing risks for mothers and infants. Overall, this study represents an important first step towards advancing our understanding of the complex relationship between Hb levels and birth outcomes and extend to more fields.

The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project aims to enhance the comprehension of nutrient biomarkers in situations involving inflammation and to produce customized assessments of anemia risk factors through collaboration between multiple agencies and countries (Suchdev et al., 2016). The BRINDA pregnancy project conducts a secondary analysis of survey data that examines various factors, including anemia, inflammation, biomarkers, and household-level risk factors, related to pregnant women. The goal is to use this study to enhance our understanding of these factors and their impact on pregnant women.

Methods

Data source

We used the pregnancy woman data from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) (Suchdev et al., 2016) to test potential methods. These 8 datasets, which were randomized clinical trials unless otherwise stated were collected in Bangladesh (2012&2013), Ghana, Gambia, Malawi, Mexico, United States (Cohort study), Vietnam. While 4 datasets only measured Hb twice at enrollment and end of gestation, another 4 datasets measured three times with one more measurement at medium gestation. Our analysis combined 8 datasets together to investigate Hemoglobin (Hb) value during pregnancy and birth outcomes. Previous study have verified low maternal hemoglobin (Hb) (<110 g/L) was associated with poor birth outcomes including PTB, LBW, and SGA. Their study examined the Hb value at different time point and applied different methods to investigate the association. (Young et al., 2019)

In the dataset, the Hb are measured in trimester and gestational weeks. We created two datasets based on both discrete and continuous measurements to examine the methods if the datasets satisfied the

requirements. For the distribution of Hb measurements during pregnancy (Figure 1A), more measurements were available in the last trimester, whereas fewer were available in 3rd trimester. We aimed to verify whether the birth outcomes of individuals with two or more Hb measurements differed from those with any available measurement. Therefore, we conducted sensitivity analyses in the supplementary material, including standard logistic regression models among individuals with at least one Hb measurement and those with at least two Hb measurements. The results showed that the odds ratios aren't different.

Before investigating the association between Hb and birth outcome overtime, we examined the time trend of Hb. Figure 1B summarized how Hb value scatter over gestational days and separated by trimester and showed that the overall trend of Hb decreased from 1st trimester to 2nd trimester and reached to the cutoff (110g/L) approximately at the medium gestation and slightly increased from 2nd trimester to 3rd trimester. Since the overall trend of Hb was non-linear, we compared the risk for adverse birth outcome among the Hb decile groups. We fitted the logistic regression model using birth outcomes as binary outcome variable and Hb decile as predictor. Figure 2 summarized the birth outcome proportion, Hb average value and the log odds in each decile group. Based on the plot, the pattern was not very clear in 1st and 3rd trimester for LBW. Overall, the risk decreased as Hb increased but there is some unusual trend in decile 4 and decile 8. But we found the Hb reached to 120 g/L and the risk kept going down and the log odds kept smaller than 0 which means decreasing the risk of LBW after decile 6. In 2nd trimester, it was clearer to see the risk decreased and after decile1, all of the log odds are smaller than 0 and reached to the minimum when Hb reached to 110. For preterm, overall, the risk kept decreasing as Hb increased in 1st and 2nd trimester but remain stable for Hb change in 3rd trimester.

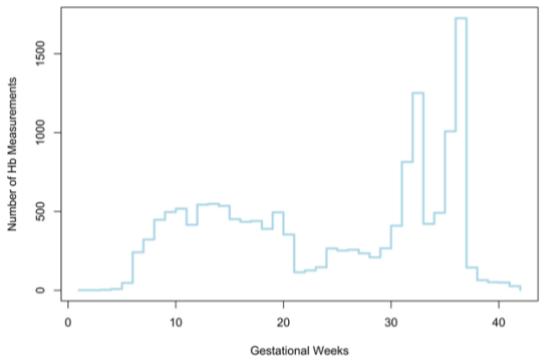

Figure 1A Histogram for the number of Hb measurements over gestational week

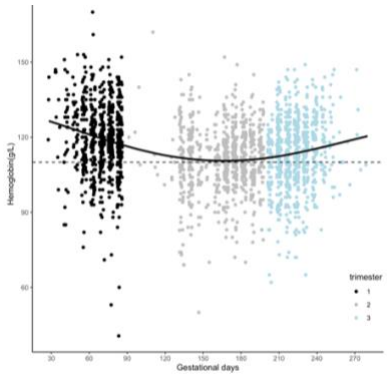

Figure 1B Scatterplots of Hb over gestational days by trimester and smooth spline between Hb and gestational days

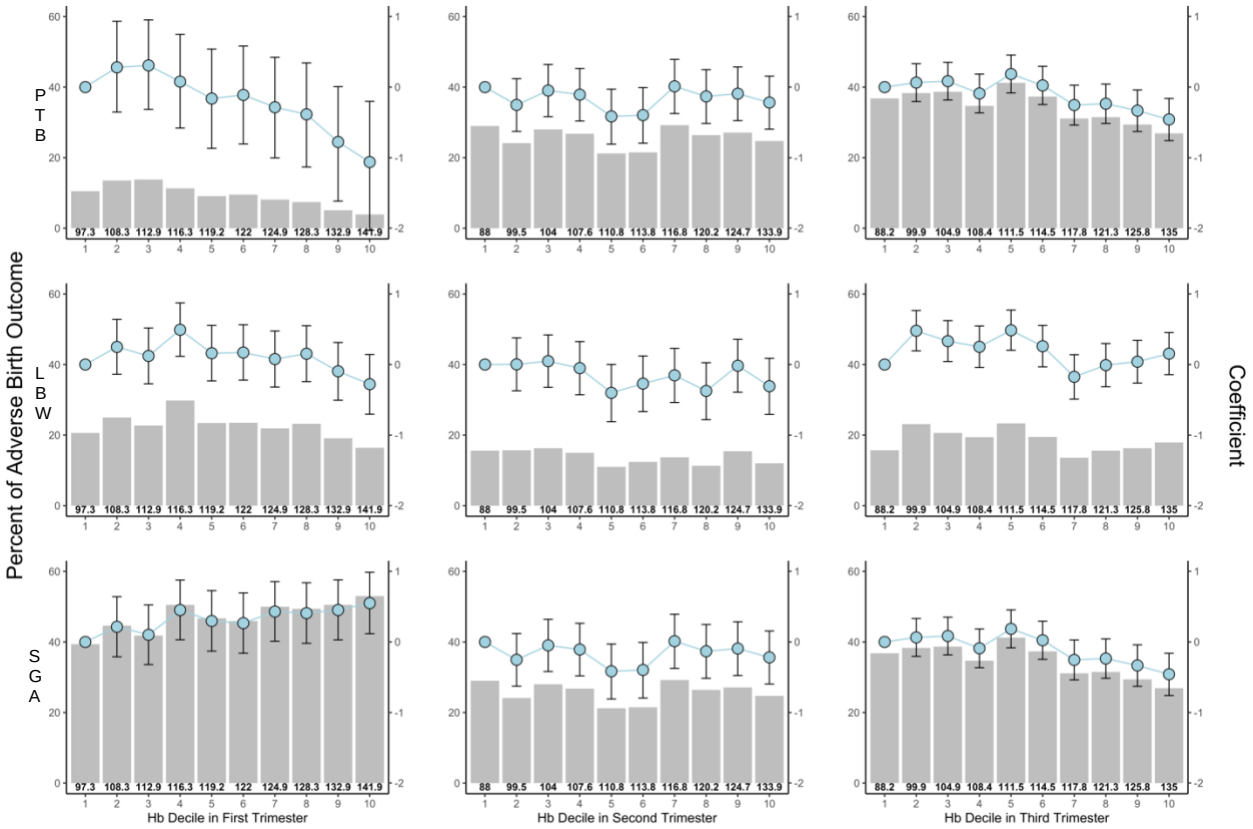

Figure 2 Proportion of adverse birth outcomes (Left y-axis) and coefficient with 95% confidence interval in simply logistic regression (Right y-axis) by decile of Hb, the number above the x-axis representing the average Hb in each decile group.

We were interested in adverse birth outcomes including preterm birth (PTB) defined as delivery before 37 weeks completed gestation (Institute of Medicine Committee on Understanding Premature & Assuring Healthy, 2007); low birth weight (LBW)defined as a birth weight of less than 2500 g (up to and

including 2499 g) (Brämer, 1988); and small for gestational age (SGA) defined as a birth weight of less than 10th percentile for gestational age (Battaglia & Lubchenco, 1967). We used INTERGROWTH-21st standard (Villar et al., 2014) for SGA quantile. Among 6456 mothers in the datasets, 313 (7.18%) delivered preterm infants; 1175 (18.6%) delivered infants with LBW; and 1456 (34.8%) delivered infants with SGA. Figure 3 shows the overlapping of adverse birth outcomes: infants with LBW are more likely to have preterm and SGA, while there are few infants with SGA and preterm simultaneously and 45 mothers delivered with all adverse outcomes.

Our final analysis cohort included individuals with at least two Hb measurements and at least one outcome available. Table 1 summarizes 6456 participants' characteristics based on the number of Hb measurements available.

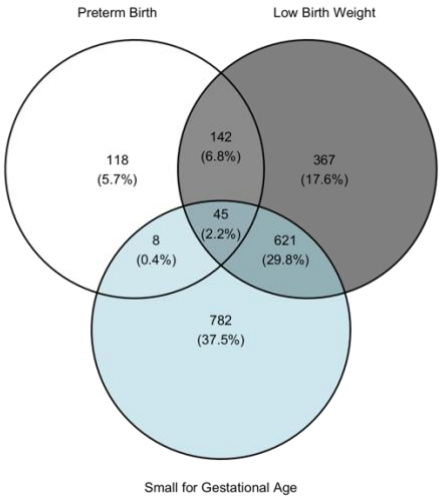

Figure 2 The overlapping of three birth outcomes in N (%)

Table 1 Distribution of study population characteristics by hemoglobin availability: Mean (SD) or N (%)

	2 measurements of Hb (N=5781)	**3 measurements of Hb (N=675)**	**Overall (N=6456)**
Age(years)	25.1 (5.81)	26.5 (4.93)	25.2 (5.72)
BMI value before pregnancy			

	24.5 (4.33)	23.0 (4.65)	24.5 (4.34)
Total number of births, including live births and stillbirths	1.96 (1.57)	3.14 (2.24)	2.02 (1.63)
Low Birth Weight			
No	4530 (79.9%)	621 (94.7%)	5151 (81.4%)
Yes	1140 (20.1%)	35 (5.34%)	1175 (18.6%)
Preterm Labor			
No	3874 (92.7%)	171 (96.6%)	4045 (92.8%)
Yes	307 (7.34%)	6 (3.39%)	313 (7.18%)
Small for gestational age			
No	2621 (65.1%)	105 (66.5%)	2726 (65.2%)
Yes	1403 (34.9%)	53 (33.5%)	1456 (34.8%)

Notation

For each subject i where i=1, ..., N, assume X_{ij} (j = 1, 2, 3) are the measurements of Hb at visit j and Y_{ik} (k = 1, 2, 3) denotes the binary outcomes: Preterm, LBW and SGA respectively. Each visit is denoted occurring at time t_{ij} measured in units of gestational weeks, or at time T_{ij} measured in trimester. Z_{ij} are the vector of covariates. Since some subjects do not have all three measurements, we will let n_i denote the number of Hb measures available per subject.

Statistical Methods

There are several methods available to evaluate the association between biomarkers during pregnancy and birth outcomes. Four standard methods based on logistic regression are commonly used: (1) fitting separate simple logistic regressions for each time window using the exposure in each window as the main predictor, (2) using the average exposure value as the main predictor in a simple logistic regression, (3) using the maximum or minimum exposure value across measurements as the main predictor in a simple logistic regression, and (4) using all measurements as predictors in a multiple logistic regression. However, these methods do not consider the relationship between repeated measurements, changes in exposure over time, or how changes in exposure are associated with birth outcomes. Another approach could address the multi-collinearity of these repeated measurements is to

obtain the conditional value (Addo et al., 2013) as the predictor, the individual-specific residual calculated from the logistic regression of exposure value at each time window on previous exposure measurements. We are particularly interested in exploring the effect of exposure trajectory on outcomes in longitudinal data. The distributed lag model (Gasparrini, Armstrong, & Kenward, 2010) is a powerful method that can account for exposure trajectory and find a sensitive window to estimate the time-varying association between the probability of outcome and exposure level. The two-stage mixed-effects model can acknowledge the individual-specific exposure path while also considering the exposure pattern. The generalized additive mixed model specifies a smooth function of time and evaluates the effect based on individual path by reversing the dependent and independent outcomes. In each segment of our results, we explored the above methods in the application of longitudinal data and illustrate them using example data. We have provided R codes for each method in an additional file.

Results and Discussion

Standard Methods

(1) Parallel logistic models by trimester

$$\text{logit}[\Pr(Y_{ik} = 1)|X_{ij})] = \beta_{0j} + \beta_j X_{ij} \text{ for } j = 1, \dots, n; k = 1, 2, 3$$

(2) Model using average exposure across visits as a summary

$$\text{logit}[\Pr(Y_{ik} = 1)|X_{ij})] = \beta_0 + \beta\bar{X}_i, k = 1, 2, 3 \quad \text{Where } \bar{X}_i = \frac{1}{n_i}\sum_{j=1}^{n_i} X_{ij}.$$

(3) Model using acute exposure value across visits as a summary

$$\text{logit}[\Pr(Y_{ik} = 1)|X_{ij})] = \beta_0 + \beta X_{i,\ \max/\min}, k = 1, 2, 3$$

(4) Multivariable logistic regression

$$\text{logit}[\Pr(Y_{ik} = 1)|X_{ij})] = \beta_{0j} + \sum_{j=1}^{n} \beta_j X_{ij} \text{ for } j = 1, \ldots, n; k = 1, 2, 3$$

Figure 1 presented the odds ratio and 95% confidence interval for the four standard logistic regression methods and the multiple logistic regression method. The odds ratios were calculated for a 5-unit increase in Hb value, as the Hb values were divided by 5 for everyone to obtain a meaningful estimate. The results showed that Hb values in 1st and 2nd trimester were associated with a decreased risk of preterm birth, with odds ratios of 0.89 (95% CI: 0.84, 0.95) and 0.92 (95% CI: 0.87, 0.98), respectively. However, the Hb value in 1st trimester was associated with an increased risk of SGA, with an odds ratio of 1.05 (95% CI: 1.01, 1.09). On the other hand, the Hb value in 3rd trimester was associated with a decreased risk of SGA, with an odds ratio of 0.95 (95% CI: 0.93, 0.97). The associations between the average Hb value and the three birth outcomes were not significant. The minimum Hb value during pregnancy was also not significantly associated with adverse birth outcomes. However, a higher maximum Hb value was significantly associated with a decreased risk of preterm birth, with an odds ratio of 0.95 (95% CI: 0.92, 0.98). In the multiple logistic regression analysis, a higher Hb value in 2nd trimester was significantly associated with a decreased risk of SGA, with an odds ratio of 0.92 (95% CI: 0.87, 0.98). Overall, these results suggested that the association between Hb value and birth outcomes may vary depending on the trimester of. Additionally, the different methods used to analyze the longitudinal data can lead to different conclusions, highlighting the importance of choosing an appropriate method for the specific research question.

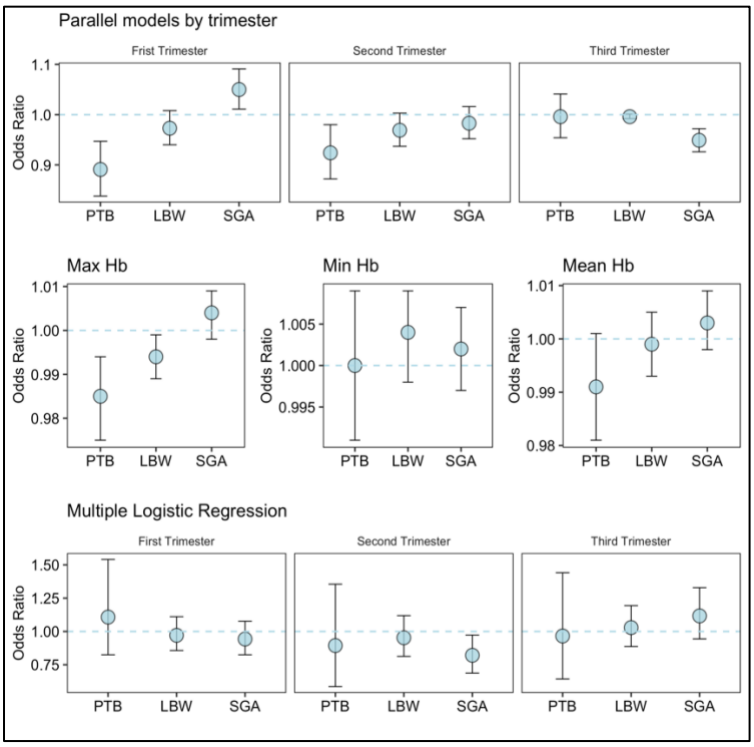

Figure 4 The odds ratio and 95% confidence interval of 5-unit Hb increasing and adverse birth outcome in each standard logistic regression method

A simple statistical approach to identify the sensitive window is to fit n separate regression models for each measurement. This model actually doesn't account for the trajectory of Hb and the effect of Hb change at all. It only focuses on the association between a single measurement in a specific window and the outcome. Therefore, while the parallel logistic model may help identify a sensitive window over time, it is limited for estimating the effect of trajectory over time. A straightforward approach to summarize a longitudinal data is to calculate the average level over time as the main predictor. This approach could use all individuals in the analysis since the available measurements will be considered as representative. The drawback is that only accounting for the average level can't investigate the sensitivity window or the effect of exposure pattern. Therefore, this approach may be useful when the outcome is not associated with exposure pattern even the exposure is measured longitudinally, and the aim is to look at the overall level. Another simple approach is to regress the dependent variable on the extreme value, either maximum or minimum. This model has an assumption that the outcome is more likely to depend on the extreme value instead of the overall or development of exposure when is applied to longitudinal data. The

simplest way to summarize the information from all measurements is to include them in the same model. While this model is easy to understand and use, it requires the complete visit and causes a big problem, the collinearity. Based on the dataset, the correlation between Hb in 1st and 2nd trimester was 0.43; the correlation between Hb in 2nd and 3rd trimester was 0.55; the correlation between Hb in 1st and 3rd trimester was 0.39. The multi-collinearity will lead to an inaccurate estimate and inflated variance which is meaningless for effect estimation.

Logistic regression using conditional Hb

To address collinearity and the corresponding inflated variances, one potential approach is to include the conditional exposure value in the model. We calculated the conditional exposure value by regressing the exposure value on the previous exposure and extracting the residuals from the regression model. Consequently, conditional exposure values are uncorrelated with each other. Each conditional exposure can be interpreted as a deviation from the expected value in the context of the mean change pattern in the population.

In the example dataset, we used Hb in 1st trimester as the anchor and incorporated it as a predictor in the final regression model. First, we calculated the conditional Hb in 2nd trimester as the residuals from a model regressing the Hb in 2nd trimester on the Hb in 1st trimester. We also obtained the conditional Hb in 3rd trimester as the residuals from a model regressing the Hb in 3rd trimester on the Hb in 1st trimester and Hb in 2nd trimester. Second, we fitted a multiple logistic regression model with three predictors: Hb in 1st trimester, the conditional Hb in 2nd trimester, and the conditional Hb in 3rd trimester. After addressing the collinearity problem, we detected a significant protective effect of a higher conditional Hb value in 2nd trimester on SGA. With a 1 deviation increase from the population mean of Hb in 2nd trimester, the odds ratio for SGA was 0.972 (95% CI: 0.945, 0.998).

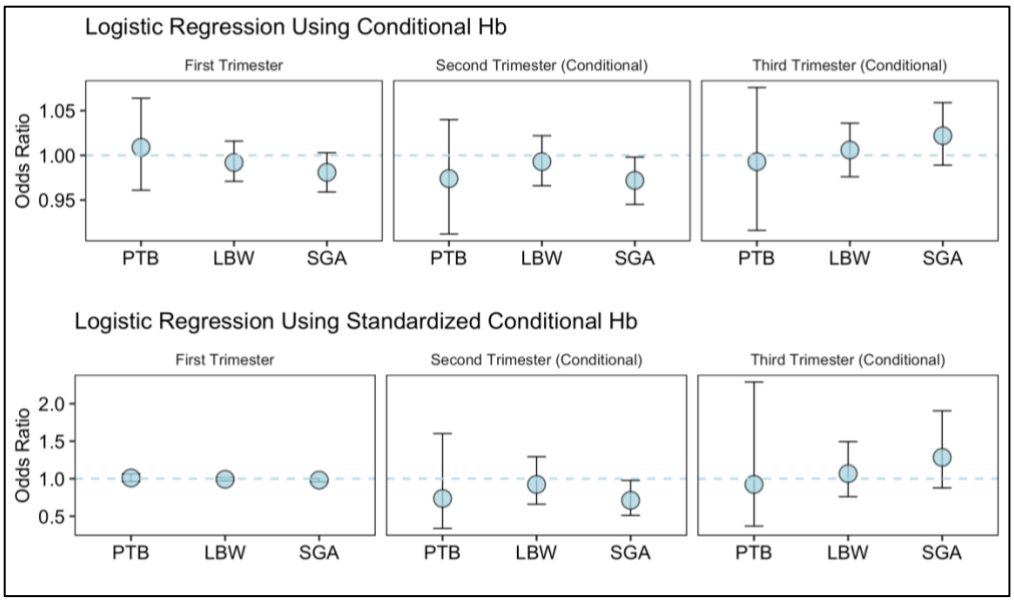

Figure 5 The odds ratio and 95% confidence interval of 1 Standard deviation increasing in Hb and adverse birth outcome

Using logistic regression based on the conditional exposure enables incorporation of all measurements in one model and solves the problem of multicollinearity. As a result, this approach can provide relatively accurate effect estimates for each measurement by controlling for all other measurements. However, this approach still focuses on a single measurement and does not account for how changes in exposure over time may affect the outcome.

Distributed lag non-linear model

This method aims to estimate the time-varying association between the probability of outcome and the exposure at a given time by constructing an exposure lag space throughout the time period. The focus is on a function (Gasparrini, 2014) that describes the dependency in terms of the exposure history evaluated at a given time window. Specifically, the logistic regression model is based on a constrained structure of coefficients that represents the effect of each time point. We fit constrained DLNM that assumes these effects are a smooth function of time. To create an individual-specific exposure profile accounting for both exposure path and lag effect, we assume a specific effect within lag intervals and a standard exposure–response function. The next step is to use the exposure profile as predictors in the logistic regression to obtain the effect estimates.

$$\mathbf{logit}[\mathbf{Pr}\ (\mathbf{Y_{ik}} = \mathbf{1})|\mathbf{X_{ij}})] = \boldsymbol{\beta_0} + \sum_{\mathbf{j=1}}^{\mathbf{n}} \boldsymbol{\alpha_j}\, \mathbf{X_{ij}}$$

where $\boldsymbol{\alpha_j}=\boldsymbol{h(j)}$, a smooth function of time

In the example dataset, we extended the Hb value in each trimester to corresponding gestational weeks. Firstly, we assumed that the Hb of one individual during gestational weeks followed either a linear or spline function, and we used the same function for the lag effect to obtain the exposure profile and fitted the model. To select the appropriate functions for exposure and lag effect, we examined different options and selected the model based on AIC values for preterm, LBW, and SGA outcomes separately. The summary of the model selection process was presented in Table 3. Secondly, we defined the reference value of Hb as 100 and calculated the odds ratio for a 5-unit increase from this reference value to visualize the effect at each gestational week. We identified a sensitive window as a period where the pointwise 95% confidence interval did not include zero. Our analysis revealed a sensitive window for decreasing the risk of SGA between gestational week 1-28 with a 5-unit increase in the Hb value from the reference level of 100.

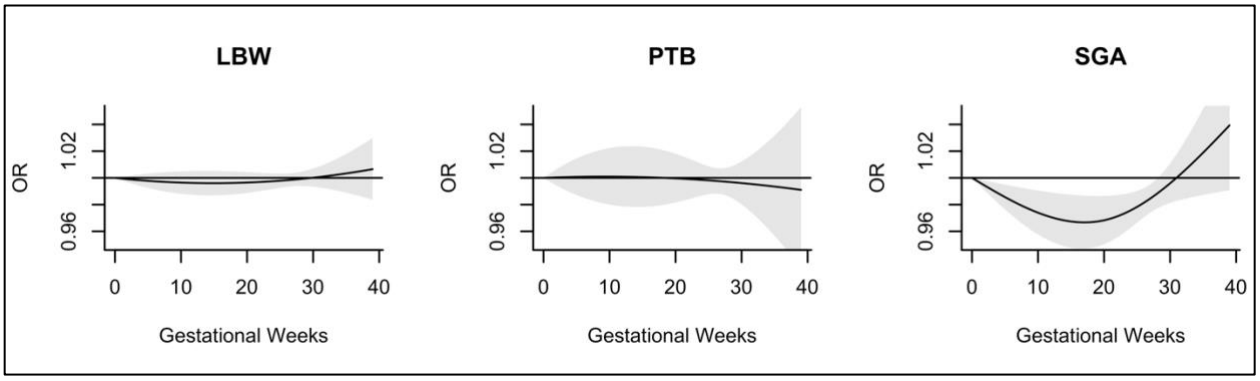

Figure 6 Association between weekly Hb levels over gestation and adverse birth outcomes. This figure demonstrates the association between Hb over gestation and adverse birth outcomes using a distributed lag model assuming week-specific effects. The y-axis shows the odds ratio (OR) of adverse birth outcomes in relation to a 5 increase in Hb; the x-axis depicts gestational age in weeks. The solid line shows the predicted OR, and the gray area indicates the 95% confidence interval.

DLNMs can simultaneously describe non-linear exposure–response relationships and lag structures by specifying appropriate functions. They are commonly used to model the effect of a predictor variable on a

response variable over time, while accounting for the possible lag effects of the predictor variable. In conclusion, DLNM is a valuable tool for capturing non-linear relationships, lagged, and time-varying effects between exposure and outcome. This is particularly important for understanding how the effects of a predictor variable may change over time. However, the results obtained from DLNM are dependent on strong assumptions about the shape of the non-linear relationship between exposure and outcome, as well as the distribution of lags. Violations of these assumptions can lead to biased results. Additionally, DLNM focuses more on how the effect of exposure on the outcome changes over time and lacks information about different exposure levels and changes in exposure. One of the challenges of applying DLNM is that it requires a complete dataset with a large amount of data for accurate estimation, especially when the model includes many predictor or lag terms. This may limit its applicability in situations where data is limited or unavailable.

Two-stage mixed effects model

The two-stage approach (Chen, Ferguson, Meeker, McElrath, & Mukherjee, 2015) can address the longitudinal trend that is relevant in terms of capturing the association between exposure and outcome. In stage 1, the time-varying exposure is modeled on time. This step may consider the random slopes and intercepts (or random intercepts only based on the individual trajectories). In stage 2, the estimated random effects of each individual are extracted from stage 1 and used as predictors to fit a logistic regression model:

$$\boldsymbol{Stage\ 1}: \boldsymbol{X_{ij} = a_{0i} + a_0 + a_{1i}t_{ij} + a_1 t_{ij} + \epsilon_{ij}}$$

$$\boldsymbol{Stage\ 2}: \boldsymbol{logit}[\mathbf{Pr}\,(\boldsymbol{Y_{ki} = 1})|\boldsymbol{a_{0i}, a_{1i}})] = \boldsymbol{\beta_0 + \beta_1 a_{0i} + \beta_2 a_{1i}}$$

Where $\mathbf{a_{0i}}$ and $\mathbf{a_{1i}}$ are random intercept and slope which presents the individual specific initial value and longitudinal trajectory of exposure. $\mathbf{a_0}$ and $\mathbf{a_1}$ are fixed effects and $\mathbf{t_{ij}}$ are the time point for each participant. $\boldsymbol{\epsilon_{ij}}$ is the random error term which is normally distributed with mean equal to 0.

In our example analysis, we regressed the Hb on trimester in stage 1 which used 1st trimester as reference group and on gestational weeks which we treated as continuous. In stage 2, we extracted the individual specific random intercept and slope and fitted logistic regression model for 3 birth outcomes. The results were summarized in Table 2.1 and Table 2.2. As for how the Hb changes over time, the Hb significantly decreased with gestation development. For the intercept, which could be regarded as the Hb at the start of gestation, individuals with higher intercept are less likely to have LBW and preterm when modeled the birth outcomes on trimester.

Table 2.1 The association between gestational week or trimester and hemoglobin

Predictors	**Fixed Effect**	**Coefficients**	**95% CI**
Gestational Age **(**Week)	Intercept	118.60	(118.12, 119.07)
	Slope	-0.19	(-0.21, -0.18)
Trimester	Intercept	120.53	(120.10, 120.96)
	2nd Trimester Slope	-8.67	(-9.19, -8.14)
	3rd Trimester Slope	-7.81	(-8.26, -7.35)

Abbreviations: CI, confidence interval

Table 2.2 The association between the random effect from Stage I and the risk of birth outcomes

Outcome	**LBW**		**PTB**		**SGA**	
Random Effect	OR	95% CI	OR	95% CI	OR	95% CI
Intercept (Hb)	1.000	(0.991, 1.01)	1.018	(0.999, 1.036)	1.006	(0.997, 1.016)
Gestational Age (Week) Slope	1.595	(0.669, 3.801)	1.282	(0.269, 6.103)	0.106	(0.043, 0.258)***
Intercept (Hb)	0.986	(0.976, 0.997)**	0.997	(0.996, 0.998)***	1.008	(0.998, 1.019)
2nd Trimester slope	0.997	(0.970, 1.024)	1.000	(0.997, 1.004)	0.992	(0.964, 1.019)
3rd Trimester slope	1.011	(0.986, 1.036)	1.006	(1.003, 1.009)***	0.926	(0.902, 0.950)***

Abbreviations: OR, odds ratio; CI, confidence interval

Using this method and considering gestational age in week for slope, Hb exhibits evidence suggesting the individual specific predicted slope significantly decreased the risk of SGA. When considering trimester for slope, the individual specific predicted intercepts was significantly associated with preterm (OR = 0.997, 95% CI: (0.996, 0.998)) and LBW (OR = 0.986, 95%CI: (0.976, 0.997)). The estimated effect for predicted slope in 3rd trimester was also significantly associated with preterm birth (OR = 1.006, 95% CI: (1.003, 1.009) and SGA (OR = 0.926, 95% CI: (0.902, 0.950)) while for predicted slope in 2nd trimester was not statistically significant. We can interpret the results as that the mean Hb level, as represented by the individual specific predicted intercepts, was significantly associated with preterm birth and LBW when adjusting the trends of Hb in trimester across pregnancy. Also, the trends of Hb in 3rd trimester compared to 1st trimester contributed to preterm birth and SGA. However, the random intercepts and slopes in stage 1 were high correlated both for gestational age in week and trimester which caused the problem of collinearity.

The two-stage mixed effects model accounts for both fixed and random effects and gives estimated effect for how individual-level exposure change over time, while accounting for fixed effect and individual-specific variation, when repeated measurements are taken on the same individuals over time. Also, it can handle missing data. This approach can address the non-linearity problem by including smooth functional of Hb which summarized the trajectory in stage 1 or including higher-order terms or curvature characteristics as predictors in Stage 2, when the trend for exposure is non-linear in stage 1.

Generalized additive mixed model

This approach reverses exposure and outcome to fil the model in which the linear exposure depends linearly on unknown smooth functions of time and outcomes as time-invariant binary predictors. We used the generalized additive mixed model (GAMM) (Wood S and Scheipl F, 2016) as follows:

$$\mathbf{X_{ij} = \beta_0 + b_{0i} + f_1(t_{ij}) + f_2(t_{ij})Y_{ik} + \varepsilon_{ij}}$$

Where β_0 is the fixed intercept and b_{0i} is the corresponding random intercept and $f_1(t_{ij})$ and $f_2(t_{ij})$ are smooth functions of time, gestational age at Hb measurements. This approach takes the longitudinal trend of exposure into account for the association with outcomes parametrically or non-parametrically. The model also incorporates an interaction term between smooth function of time and outcome which can estimate different smooth function curve for different outcomes.

In the example dataset, we fitted the model with the unconstrainted smooth functions and then limited the degree of freedom associated with smooth functions to avoid overfitting and adjust the trajectory to common U shape (Dewey & Oaks, 2017). Next step, we calculated the predicted Hb value by gestational age in mothers with adverse birth outcome and without it based on the estimated smooth functions for preterm birth, LBW, and SGA respectively. The estimated degree of freedom (EDF) for the difference between the 2 curves was 4, 2 and, 2.5 for preterm birth, LBW and SGA, indicating a linear difference in two groups for LBW across gestation but a non-linear difference for preterm birth and SGA. As shown in Figure 5, individuals with adverse outcomes tended to have lower Hb generally across gestation but the difference is not significantly except in 1st trimester for preterm birth.

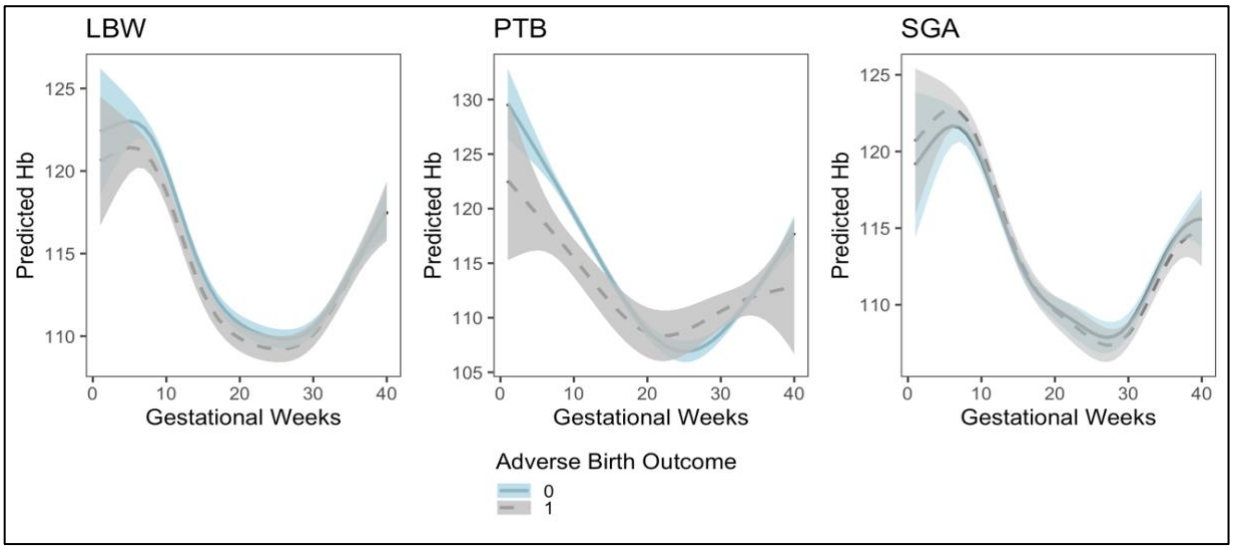

Table 7 Predicted Hb and 95% confidence intervals over gestational age in week from generalized additive mixed models (GAMM) in pregnancy women with adverse birth outcomes (dashed) compared to normal birth outcomes (solid).

This approach is quite flexible to use since it doesn't assume that the exposure has a linear relationship with the outcome or that the residuals are normally distributed. Therefore, it can address the change in exposure over time as well as interaction terms. While GAMM can relax the assumption and is easy to implement, it can't give a specific estimate for the effect. Also, interpreting the model output can be challenging. Another problem is that GAMM will overfit the data if the model is too flexible i.e., the degree of freedom is too high, or the sample size is too small.

Conclusion:

In conclusion, by applying to examine the association between maternal Hb during pregnancy and birth outcomes, we illustrated 7 statistical methods that can be utilized to longitudinal analysis. The distributed lag non-linear model, two-stage mixed effect model and generalized additive mixed model are preferred when investigators are interested in the exposure pattern. GAMM emphasizes visualization while others could provide effect estimations. Using BRINDA pregnancy women dataset, we have a bigger sample size to implement in our example analysis to fully illustrate the approach without the limitation of sample size. Also, BRINDA collected the data from 8 countries, and it gives us the potential opportunity in the future work to investigate the interaction between the association and sample characteristic. Based on the existing methods (Chen et al., 2015), we added one more useful model – the distributed lag non-linear model, which can estimate a time-specific effect and detect the exposure-lag-response association. By illustrating the application in modeling birth outcomes on Hb, this study provides extremely useful insight into the pros and cons of statistical methods modeling time-varying biomarkers or any other predictors in association with not time-varying birth or pregnancy outcomes. The methods can be applied to a larger sample size analysis which will contribute to improve the surveillance and research on policies and programs related to micronutrient nutrition in public health.

Table 3 summarizes the strengths and limitations of each method. One can find a more suitable approach for a specific situation accounting for the research aim, data availability, and characteristic of the biomarker measurement. Therefore, these methods are useful for researchers especially in nutrition

area to identify a sensitive window of biomarker for outcomes, examine the association between biomarker and outcomes or estimate the effect of biomarker on outcomes.

Table 3 Pros and cons of methods for modeling repeated biomarkers in association with a binary outcome

Method	Pros	Cons
Standard Logistic Regression	• Straightforward to use • Straightforward interpretation • Some could retain Subjects with incomplete data	• Trends of biomarker relevant to the outcome may be missed
Model using conditional value	• Minimize bias and inflated variances due to collinearity • Each conditional exposure could be interpreted as a deviation from the expected value in the context of the mean change pattern in population	• Require complete dataset • Residual depends on the overall trajectory of the population in general.
Distributed lag non-linear model	• Account for the biomarker trend • Identify a sensitive window	• Require complete dataset
Two stage mixed effects model	• Flexible modeling of biomarker pattern over time in Stage 1 • Examines effect of characteristics carried from Stage 1 in Stage 2 • Naturally accounts for between subject heterogeneity	• Uncertainty from Stage 1 is not incorporated in Stage 2 which may lead to biased results • May not be useful when biomarker are unstable over time
Generalized additive mixed model	• Accounts for longitudinal nature of exposure • Trends of biomarker can be depicted parametrically or non-parametrically for each group	• Not temporally logical • Risk cannot be estimated

References

Addo, O. Y., Stein, A. D., Fall, C. H., Gigante, D. P., Guntupalli, A. M., Horta, B. L., . . . Martorell, R. (2013). Maternal height and child growth patterns. J Pediatr, 163(2), 549-554. doi:10.1016/j.jpeds.2013.02.002

Cando, L. F. T., Dispo, M. D., & Tantengco, O. A. G. (2022). Salivary progesterone as a biomarker for predicting preterm birth: A systematic review and meta-analysis. Am J Reprod Immunol, 88(6), e13628. doi:10.1111/aji.13628

Chen, Y. H., Ferguson, K. K., Meeker, J. D., McElrath, T. F., & Mukherjee, B. (2015). Statistical methods for modeling repeated measures of maternal environmental exposure biomarkers during pregnancy in association with preterm birth. Environ Health, 14, 9. doi:10.1186/1476-069x-14-9

Conlin, W. E., Hoffman, M., Steinley, D., & Sher, K. J. (2022). Cross-sectional and longitudinal AUD symptom networks: They tell different stories. Addict Behav, 131, 107333. doi:10.1016/j.addbeh.2022.107333

Dewey, K. G., & Oaks, B. M. (2017). U-shaped curve for risk associated with maternal hemoglobin, iron status, or iron supplementation. Am J Clin Nutr, 106(Suppl 6), 1694s-1702s. doi:10.3945/ajcn.117.156075

Emmett, P. M., Jones, L. R., & Golding, J. (2015). Pregnancy diet and associated outcomes in the Avon Longitudinal Study of Parents and Children. Nutr Rev, 73 Suppl 3(Suppl 3), 154-174. doi:10.1093/nutrit/nuv053

Gasparrini, A. (2014). Modeling exposure-lag-response associations with distributed lag non-linear models. Stat Med, 33(5), 881-899. doi:10.1002/sim.5963

Gasparrini, A., Armstrong, B., & Kenward, M. G. (2010). Distributed lag non-linear models. Stat Med, 29(21), 2224-2234. doi:10.1002/sim.3940

Go, H., Hashimoto, K., Kyozuka, H., Maeda, H., Nishigori, H., Sato, A., . . . Hosoya, M. (2022). Maternal hemoglobin levels and neonatal outcomes: the Japan Environment and Children's Study. J Matern Fetal Neonatal Med, 35(26), 10472-10480. doi:10.1080/14767058.2022.2130237

Jwa, S. C., Fujiwara, T., Yamanobe, Y., Kozuka, K., & Sago, H. (2015). Changes in maternal hemoglobin during pregnancy and birth outcomes. BMC Pregnancy Childbirth, 15, 80. doi:10.1186/s12884-015-0516-1

Liu, X., An, H., Li, N., Li, Z., Zhang, Y., Zhang, L., . . . Ye, R. (2022). Preconception Hemoglobin Concentration and Risk of Low Birth Weight and Small-for-Gestational-Age: A Large Prospective Cohort Study in China. Nutrients, 14(2). doi:10.3390/nu14020271

Marvin-Dowle, K., Burley, V. J., & Soltani, H. (2016). Nutrient intakes and nutritional biomarkers in pregnant adolescents: a systematic review of studies in developed countries. BMC Pregnancy Childbirth, 16, 268. doi:10.1186/s12884-016-1059-9

Peng, Z., Si, S., Cheng, H., Zhou, H., Chi, P., Mo, M., . . . Yu, Y. (2022). The Associations of Maternal Hemoglobin Concentration in Different Time Points and Its Changes during Pregnancy with Birth Weight Outcomes. Nutrients, 14(12). doi:10.3390/nu14122542

Suchdev, P. S., Namaste, S. M., Aaron, G. J., Raiten, D. J., Brown, K. H., & Flores-Ayala, R. (2016). Overview of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. Adv Nutr, 7(2), 349-356. doi:10.3945/an.115.010215

Tous, M., Villalobos, M., Iglesias, L., Fernández-Barrés, S., & Arija, V. (2020). Vitamin D status during pregnancy and offspring outcomes: a systematic review and meta-analysis of observational studies. Eur J Clin Nutr, 74(1), 36-53. doi:10.1038/s41430-018-0373-x

Villar, J., Cheikh Ismail, L., Victora, C. G., Ohuma, E. O., Bertino, E., Altman, D. G., . . . Kennedy, S. H. (2014). International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. Lancet, 384(9946), 857-868. doi:10.1016/s0140-6736(14)60932-6

Wood S and Scheipl F. (2016). Package ‘gamm4’. Retrieved from https://cran.r-project.org/web/packages/gamm4/gamm4.pdf:

Wright, J. M., Lee, A. L., Rappazzo, K. M., Ru, H., Radke, E. G., & Bateson, T. F. (2023). Systematic review and meta-analysis of birth weight and PFNA exposures. Environ Res, 222, 115357. doi:10.1016/j.envres.2023.115357

Wu, L., Sun, R., Liu, Y., Liu, Z., Chen, H., Shen, S., . . . Deng, G. (2022). High hemoglobin level is a risk factor for maternal and fetal outcomes of pregnancy in Chinese women: A retrospective cohort study. BMC Pregnancy Childbirth, 22(1), 290. doi:10.1186/s12884-022-04636-9

Young, M. F., Oaks, B. M., Tandon, S., Martorell, R., Dewey, K. G., & Wendt, A. S. (2019). Maternal hemoglobin concentrations across pregnancy and maternal and child health: a systematic review and meta-analysis. Ann N Y Acad Sci, 1450(1), 47-68. doi:10.1111/nyas.14093