Inflammatory Pathways and Micronutrient Status: Implications for Anemia in Pregnancy

Final Project

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Introduction

Anemia is a common disorder that affects over 40% of pregnancies globally.[1] As gestational age increases, nutrients from the mother must supplement the growing fetus to ensure proper organelle functionality and development. Low micronutrient accrual has previously been associated with adverse birth outcomes, including preterm birth, low birth weight, and infant mortality. Micronutrients play a critical role in maintaining homeostasis and may indirectly affect iron status. Inflammation may also play a role in the development of anemia from interference of iron-related red blood cell production and iron metabolism. C-reactive Protein (CRP) is produced in direct response to inflammation in the body and has been previously utilized as a biomarker of inflammation.[2] Previous studies have examined the association between micronutrient concentration or inflammation with anemic status, but not the joint effect of these statuses. Individual relationships of ferratin and serum iron do support the causal framework that inflammation impacts several micronutrient metabolism pathways.[3] In this analysis, we examined the combined effect of micronutrients and inflammation on anemia. Specifically, 1) investigating the association between individual micronutrients and CRP in pregnant women; 2) characterizing the contributions of micronutrients and CRP together on anemic status in pregnant women; and 3) develop a prediction model for anemia.

Methods

Study Population and Design

Participants for this study were part of the National Health and Nutritional Examination Survey (NHANES) study, a publicly available cohort compiled by the Centers for Disease Control and Prevention.[4] NHANES is a national-level program of studies that are designed to assess the health and nutritional level of adults and children in the United States. We selected 480 pregnant women from the study to include. We ultimately examined four micronutrients, serum ferritin (µg/L), serum folate (nmol/L), serum iron (µmol), and serum vitamin B12 (pmol/L), along with CRP (mg/L).

Data Cleaning

We employed an exclusion criterion prior to statistical analyses. First, we removed outliers from the micronutrient and CRP data that were three standard deviations outside of the mean value (n = 30). Additionally, we removed participants with missing weight (n = 7) and marital status (n = 34). Body mass index (BMI) was calculated by dividing the weight by height, in meters, squared. Participants with hemoglobin levels under 12 g/dL were considered anemic. Micronutrient and CRP concentrations were natural log-transformed to normalize the data, reducing heteroscedasticity.

Statistical Analysis

Aim 1: We fit multivariable linear regression models to examine the association between individual micronutrients and CRP, and if gestational and maternal age modified these relationships. For each model, we first regressed the log-transformed micronutrient concentration on the log-transformed CRP. We then used partial F-tests and t-tests to determine if maternal and gestational age are modifiers by including them using ANOVAs in a stepwise procedure.

Aim 2: We fitted single- and multi-variable logistic regression models to characterize the contributions of CRP and micronutrients to anemic status, controlling for demographic and clinical covariates, including maternal age, BMI, marital status, number of pregnancies, and gestational age. We decided to only include gestational age and not trimester since these variables represent the same information and

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[1] Beckert RH, Baer RJ, Anderson JG, Jelliffe-Pawlowski LL, Rogers EE. Maternal anemia and pregnancy outcomes: a population-based study. *J Perinatol*. Jul 2019;39(7):911-919. doi:10.1038/s41372-019-0375-0

[2] Nehring SM, Goyal A, Patel BC. C Reactive Protein. *StatPearls*. 2024.

[3] Williams AM, Ladva CN, Leon JS, et al. Changes in micronutrient and inflammation serum biomarker concentrations after a norovirus human challenge. *Am J Clin Nutr*. Dec 1 2019;110(6):1456-1464. doi:10.1093/ajcn/nqz201

[4] Prevention CfDCa. About the National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/about_nhanes.htm

continuous predictors are preferred over categorical. We hypothesize that these demographic and clinical covariates may modify the effect of CRP and micronutrients on anemic status, thus including interaction terms into the base model. Our initial model includes all our micronutrients, clinical covariates, and interactions between them.

We then applied a stepwise selection model, using Bayesian Information Criterion (BIC) to assess if any of the covariates, and their respective interaction terms, significantly contribute to the model. With the number of terms in the model, BIC was used to determine significant covariates since it has a stricter inclusion criterion compared to other methods, increasing our confidence in the model.

Aim 3: Using the final model derived from Aim 2, we developed a prediction model to assess anemic status in pregnant women. To develop the prediction model, we utilized a cross-validation approach. We randomly split our data into two subsets, one training set and one validation set. We employed a stepwise selection to identify the best model based on the training set. This model was then used on the validation set to determine the performance. We assessed the performance of the model by calculating the Area Under the Receiver Operating Characteristic Curve (AUC).

Results with a p-value less than 0.05 were considered statistically significant. Analyses were conducted using R.

Results

Out of 412 pregnant women that were analyzed, 143 were anemic. The mean log-transformed concentration for CRP was 1.6 mg/L, with 1.6 mg/L for those with anemia and 1.6 mg/L for those without anemia. When comparing micronutrient the mean log-transformed concentration, hemoglobin, serum ferritin, serum folate, serum iron, and vitamin B12 was lower for pregnant women with anemia compared to those without. The mean age was 27 years old (healthy = 27, anemic = 25) with an average BMI of 29 kg/m^2. Additional summary statistics can be found in Table 1.

Aim 1

From the univariate linear regression models assessing CRP on individual micronutrients, we only found that vitamin B12 was the only micronutrient significantly associated with CRP ($p = 0.002$). When analyzing models that also contain the modifying effects of maternal age and gestational age, we found that gestational age significantly contributes to a model that already contains CRP. Our findings indicate that a one mg/L increase in log-transformed CRP would result in a -0.06 (95% CI: [-0.09, -0.02]) times change in vitamin B12 level ($p = 0.004$), controlling for gestational age. For serum ferritin and serum folate, we found that maternal age and gestational age modify the association. So, a one mg/L increase in log-transformed CRP would result in a 0.09 (95% CI: [0.01, 0.17]) and –0.20 (95% CI: [-0.06, 0.02]) times change in serum ferritin ($p =0.02$) and serum folate ($p = 0.38$), respectively. Finally, maternal age modified the association between CRP and serum iron, meaning that a one mg/L increase in log-transformed CRP was significantly associated with a –0.05 (95% CI: [-0.09, 0.00002]) times change in serum iron ($p = 0.05$).

Aim 2

In the univariate logistic regression models, we found that anemia status was significantly associated with serum ferritin ($p < 0.001$), serum folate ($p < 0.001$), serum iron ($p < 0.001$), and vitamin B12 ($p = 0.03$). When combining all the micronutrients and CRP together in one model, we see that serum ferritin, serum folate, and serum iron significantly contribute to anemia status. After adding in clinical and demographic covariates and conducting the stepwise procedure, we found that a one nmol/L increase in log-transformed serum folate would result in a -0.86 (95% CI: [-1.40, -0.32]) times change in the odds of having anemia ($p = 0.002$), holding all other covariates constant. A one µmol/L increase in log-

transformed serum iron was associated with a -1.67 (95% CI: [-2.23, -1.13]) times change in the odds of having anemia ($p < 0.001$), holding all other covariates constant. Finally, a one week increase in gestational age was associated with a 0.03 (95% CI: [0.01, 0.06]) times change in the odds of having anemia ($p = 0.007$), holding all other covariates constant. Ultimately, CRP and serum ferritin did not have a significant impact on predicting anemia status, nor did any of the interaction terms between micronutrients and clinical covariates. We used the Hosmer-Leneshow goodness-of-fit test to examine how well the model fits the data. At the $\alpha = 0.05$ level, we fail to reject the null hypothesis, concluding that there is no evidence of lack of fit in the model ($p = 0.67$).

Aim 3

We inserted a model adjusting for serum folate, serum iron, and gestational age into our prediction model. After training the initial model, we maintained the same model as the best one to predict anemia status in pregnant women. This suggests that a one nmol/L increase in log-transformed serum folate was associated with a –0.86 (95% CI: [-1.40, -0.32]) times change in the odds of having anemia ($p = 0.002$), holding all other covariates constant. Additionally, a one μmol/L increase in log-transformed serum iron would result in a –1.67 (95% CI: [-2.23, -1.13]) times change in the odds of having anemia, controlling for other covariates ($p < 0.001$).

In our prediction model, we included serum folate and serum iron as significant contributors to anemia, compared to the model from the previous aim which also included serum folate and serum iron. The AUC value of this model is 0.707 (Figure 1), indicating a moderate predictive performance of our model. This indicates that the prediction model can differentiate between pregnant women who have and do not have anemia relatively well based on their serum folate level, serum iron level, and gestational age, though there is still room for improvement.

Discussion

In this analysis, we sought to investigate the association between, CRP, micronutrients, and anemia status in pregnant women in a subset of the NHANES cohort. First, we examined the relationship between individual micronutrients and CRP, and then the combined effect on anemia status. Finally, we developed a prediction model for anemia. Additionally, we examined if maternal age, BMI, marital status, number of pregnancies, and gestational age modified the association. Overall, we found that maternal age and gestational age modified the association between micronutrients, CRP, and anemia status. Also, out of the micronutrients and CRP, serum folate and serum iron were the only ones that significantly influenced anemia status.

When looking at the combined effect of micronutrients, CRP, and clinical and demographic covariates on anemia status, we found that serum ferritin, CRP, and vitamin B12 were not significantly associated with anemia status. However, vitamin B12 and serum ferritin were significantly associated with anemia status in the univariate models. This suggests that CRP may not be the best indicator of anemia status and that the relationship between micronutrients and anemia status is independent of CRP. Finally, we were able to build a model that was moderately successful in predicting the anemia status of pregnant women based on their micronutrient levels for serum folate and serum iron, as well as their gestational age (in weeks).

Our analysis had both strengths and limitations. One notable strength of our analysis was the removal of outliers and log-transformation of our micronutrient and CRP concentrations. Doing these steps ensured that our data was normally distributed and could not be influenced by extreme values. There are a few limitations that should be addressed in our study. First, the sample size was relatively small, which could affect the generalizability of our results to the broader population of pregnant. Also, our study design was cross-sectional, which does not allow us to establish a casual pathway between micronutrient level and anemia status. Finally, we did not consider additional key sociodemographic confounders that may affect

the relationship, including socioeconomic status and dietary intake. Since we included these confounders, this may represent an overinflation of results presented.

Overall, in this study we examined the role of micronutrient level on anemia status during pregnancy. Ultimately, we found that serum folate and serum iron were the best predictive indicators of anemia status in pregnant women, and potentially general fetal development and overall maternal health during pregnancy.

Table 1. Demographic Characteristics for Participants in the NHANES survey (n = 412)

	Anemia Status		
Mean (SD)	**No (N=269)**	**Yes (N=143)**	**Total (N=412)**
C-reactive protein (mg/L)	1.6 (0.96)	1.6 (0.99)	1.6 (0.97)
Hemoglobin (g/dL)	13 (0.67)	11 (0.51)	12 (0.97)
Serum ferritin (ug/L)	2.9 (0.85)	2.4 (0.87)	2.8 (0.89)
Serum folate (nmol/L)	3.7 (0.40)	3.4 (0.47)	3.6 (0.44)
Serum iron (umol)	2.7 (0.40)	2.4 (0.48)	2.6 (0.46)
Serum vitamin B12 (pmol/L)	5.6 (0.40)	5.5 (0.41)	5.5 (0.41)
Maternal age (years)	27 (5.5)	25 (5.9)	27 (5.7)
BMI	28 (5.8)	29 (6.4)	29 (6.0)
Marital Status			
Not married, living with a partner	24 (8.9%)	13 (9.1%)	37 (9.0%)
Married	193 (71.7%)	81 (56.6%)	274 (66.5%)
Widowed	0 (0%)	1 (0.7%)	1 (0.2%)
Divorced	4 (1.5%)	0 (0%)	4 (1.0%)
Separated	6 (2.2%)	4 (2.8%)	10 (2.4%)
Never Married	42 (15.6%)	44 (30.8%)	86 (20.9%)
Pregnancy Trimester			
1st trimester	68 (25.3%)	14 (9.8%)	82 (19.9%)
2nd trimester	137 (50.9%)	97 (67.8%)	234 (56.8%)
3rd trimester	63 (23.4%)	32 (22.4%)	95 (23.1%)
Overdue	1 (0.4%)	0 (0%)	1 (0.2%)
Gestational age (weeks)	23 (10)	26 (8.0)	24 (9.4)
Number of previous pregnancies	2.6 (1.5)	2.7 (1.7)	2.6 (1.5)

Table 2. Associations between CRP and micronutrients in pregnant women

	Coef.	**p-value**	**95% CI**
Serum ferritin [a]	0.09	0.02	(0.01, 0.17)
Serum folate [a]	-0.2	0.38	(-0.06, 0.02)
Serum iron [b]	-0.05	0.05	(-0.09, 0.0002)
Serum vitamin B12 [c]	-0.06	0.004	(-0.09, -0.02)

[a] adjusted for maternal age (years) and gestational age (weeks)
[b] adjusted for maternal age (years)
[c] gestational age (weeks)

Table 3. Final Model using Bayes Information Criterion (BIC)

	Coef.	p-value	95% CI
Final Model			
Serum folate	-0.86	0.002	(-1.40, -0.32)
Serum iron	-1.67	< 0.001	(-2.23, -1.13)
Gestational age (weeks)	0.03	0.007	(0.01, 0.06)

Figure 1. ROC Curve