Philippine Journal of Science
148 (1): 167-177, March 2019
ISSN 0031 - 7683
Date Received: 02 May 2018

Determining Significant Predictors of Blood Iron Concentration and Status in Pregnant Filipino Women Using Linear Models

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The decrease in blood iron levels during pregnancy is a concern that needs to be addressed, especially among Filipinos where anemia prevalence is relatively high. This study assessed maternal age, gestational age, height, body weight during pregnancy, daily consumption of iron supplements or multivitamins, gravidity, parity, and estimated monthly household income as potential predictors of levels of hemoglobin (Hb), hematocrit (Hct), serum ferritin (SF), serum iron (SI), total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and transferrin saturation (TSAT) among 109 pregnant women residing in Quezon, Palawan using simple linear regression (SLR). Significant predictors were then incorporated into full models using multiple linear regression (MLR) following the hierarchical method. Results show that gestational age significantly contributed to predicting levels of blood iron ($p < 0.05$). Gestational age was negatively associated with Hb, Hct, SF, log_{10} SF, log_{10} SI, and TSAT but positively associated with TIBC and UIBC. Gestational age accounted for variations ranging from as low as 4% in log_{10} SI up to 35% in UIBC. Additionally, weight was positively associated with Hb ($p = 0.016$) and Hct ($p = 0.027$), parity was negatively associated with log_{10} SF ($p = 0.031$), and daily consumption of iron or multivitamin supplements was negatively associated with TIBC ($p < 0.001$) and UIBC ($p < 0.001$). These identified predictors can be used in the clinical settings to target high-risk women for treatment or intervention.

Key words: anemia, blood iron levels, gestational age, iron deficiency, linear models, pregnant Filipino women

INTRODUCTION

The public health impact of anemia due to iron deficiency is often highlighted in its implications for obstetrics and perinatal care. Iron deficiency anemia (IDA) accounts for 75% of all types of anemia that occur during pregnancy (Horowitz *et al.* 2013), and pregnant women are among the high-risk groups due to the competition on iron demands between the mother and her developing fetus (NHLBI 2014; WHO 2017a). This demand for iron can also be aggravated by other related conditions such as uterine or placental bleedings, gastrointestinal bleedings, and peripartum blood loss (Breymann 2015). IDA poses an increase in the risk of preterm delivery, perinatal mortality, low birth weight, neonatal mortality, lactation failure, and postpartum depression, among others (Abu-Ouf & Jan 2015; Di Renzo *et al.* 2015). This premise prompted

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the public health experts to require approximately 1,000–1,200 mg of iron throughout the course of pregnancy, which should be met through a balance between daily iron intake and inherent iron stores in the body of a pregnant woman (Brannon & Taylor 2017).

Anemia remained to be a public health concern in the Philippines over the last decade. Anemia in a national estimate was highest and considered a moderate public health problem in 1998 (30.6% recorded national prevalence) and continuously declined to mild public health problem in 2008 (19.5%) to 2013 (11.2%) based on the National Nutrition Surveys (NNSs) conducted by the Food and Nutrition Research Institute (FNRI) of the Department of Science and Technology (DOST) (WHO 2017a; FNRI 2015). Recently, anemia among pregnant Filipino women is at 24.6% (FNRI 2015). As a developing country, poverty, malnutrition, and famine may primarily cause anemia with iron deficiency among pregnant Filipino women. Beyond the dietary origin such as dietary habits, low intake, and low bioavailability of iron, IDA in the Philippines can be viewed as a result of complex factors that are inherent in developing countries (Florentino & Guirriec 1984).

Despite the public health implications of poor iron status, few studies have assessed the predictors of iron concentration and status to date. Furthermore, no recent study has been conducted in the Philippines that determined the factors or variables associated with and significantly contributing to anemia, iron deficiency, and IDA in pregnancy. Thus, this study determined the variables that can be used to predict the blood iron levels of a group of pregnant Filipino women residing in Quezon, Palawan. Specifically, this study assessed maternal age, gestational age, height, body weight during pregnancy, daily consumption of iron supplements or multivitamins, gravidity (*i.e.*, number of times a woman has been pregnant), parity (*i.e.*, number of times a woman has given birth to a fetus with a gestational age of at least 24 weeks), and estimated monthly household income as potential predictors of Hb, Hct, SF, SI, TIBC, UIBC, and TSAT using linear models for regression. The consistent, relatively high prevalence of anemia among pregnant Filipino women signifies the important roles of some factors that significantly contribute to the decrease in blood iron concentration and status during pregnancy. Identifying the predictors and estimating their relative contributions will be helpful in alleviating the burdens to some extent as such may be used in the clinical settings in targeting high-risk pregnant women for treatment or intervention. For instance, the implementation of more intensive, assertive, and sustainable public health policy actions such as availability of iron supplements and iron-fortified foods for daily consumption may help combat anemia during pregnancy in the Philippines.

MATERIALS AND METHODS

Study Design, Study Population, and Ethical Considerations

The study was part of a program of the DOST-FNRI targeted to a group of pregnant Filipino women living in the municipality of Quezon, Palawan. The program evaluated a nutrition strategy that aimed to improve the nutritional status of women during pregnancy. For this study, a cross-sectional design was followed and a non-probability, purposive homogeneous sampling was employed. The study protocol was reviewed and given clearance by the FNRI Institutional Ethics Review Committee (FIERC) under FIERC Registry No. 2012-09-28-0010-2. Participants submitted duly signed written informed consents, and all information obtained from the study were treated with utmost confidentiality.

A group of pre-identified pregnant women residing in four districts or *barangays* in Quezon, Palawan (Alfonso Bliss, Alfonso Poblacion XIII, Isugod, and Panitian) were recruited to join the study. They were screened based on the following inclusion-exclusion criteria: (1) should be a Filipino, (2) may or may not be taking up iron supplements and/or multivitamins, (3) assessed as healthy at the time of data collection, and (4) without disease or disorder that may alter blood iron concentration and status (*e.g.*, recent or current infection, kidney disease, chronic inflammatory disease, thalassemias or other forms of hemoglobinopathies).

A more detailed information on the study design, study population, and ethical considerations has been presented previously (Timoteo *et al.* 2018).

Measurement of Hematological and Biochemical Parameters and Predictors

The parameters used to assess blood iron levels are the outcome/dependent variables in the study. Collection of venous blood samples and measurement of the levels of Hb, Hct, SF, SI, and TIBC, as well as computation of UIBC and TSAT, were reported in detail in a previous paper (Timoteo *et al.* 2018). Briefly, Hb and Hct were measured in the field following the cyanmethemoglobin method (ICSH 1978) and the microhematocrit method (NCCLS 2000), respectively. SF was measured using a kit-based solid-phase immunoradiometric assay, while SI and TIBC were determined by the Medical Research Laboratory of the Philippine General Hospital (MRL-PGH) using a photometric assay kit. UIBC was calculated as the difference between TIBC and SI value, and TSAT was computed as: TSAT = SI / TIBC x 100%. The morphological abnormalities of red blood cells (RBCs) were also evaluated through a microscopic examination of peripheral blood smears in order to screen for inherited genetic hemoglobin disorders that may cause anemia among participants (*i.e.*, hemoglobinopathies).

Cut-offs from the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) were used to describe and classify the iron status of participants. Based on WHO (2017a), anemia was defined as: Hb<11 g/dL, Hct< 33%. On the other hand, CDC (1998) defines anemia by taking into consideration the stage of pregnancy of participant: Hb < 11 g/dL, Hct < 33% for women in 1st and 3rd trimesters; Hb < 10.5 g/dL, Hct < 32% for women in 2nd trimester. WHO (2011b) defines iron deficiency as SF < 15 ng/mL, while CDC (1998) classifies iron deficiency as SF < 12 ng/mL. TSAT < 16% also suggests iron deficiency among pregnant women (CDC 1998). The reference range for SI and TIBC (0.6–1.6 mg/L and 2.51–3.63 mg/L, respectively) were set by the MRL-PGH following the methods of CLSI (1998). The incidence of IDA, iron deficiency without anemia, and anemia without iron deficiency were estimated using Hb and SF values.

Information pertaining to the socio-demographic characteristics, current pregnancy, and obstetric history of participants were obtained through a face-to-face interview during screening. The questionnaire used was pre-tested among a group of pregnant women in Lower Bicutan, Taguig City, Metro Manila prior to data collection. Standard measurement of the height and weight of participants were also described previously. Classification of the weight-for-height values was based on the recommended weight-for-height table by month of pregnancy for Filipinos (Magbitang *et al.* 1988).

Data Processing and Statistical Analyses

Data processing and statistical analyses were carried out using IBM® SPSS® Statistics version 23. Descriptive statistics were used to characterize the study population. Continuous variables were expressed as mean ± SD (range), while categorical data were presented in frequencies n (% based on N). Prior to analyses, normality of continuous data was assessed using the Shapiro-Wilk test, skewness and kurtosis, and visual inspection of histogram plots. Log_{10} transformation was applied to positively skewed, non-normal data (*e.g.*, SF, SI, and estimated monthly household income). Pearson correlation coefficient determined the strength and direction of association between each outcome variable and continuous predictors (gestational age, maternal age, height, body weight, gravidity, parity, and estimated monthly household income), and values were interpreted following the guidelines provided by Cohen (1992). Scatterplots and residual plots were produced as initial checks to detect potential outliers and assess linearity and appropriateness of linear model for the data. Upon checking the assumption of linearity between variables, SLR determined whether each independent variable significantly predicts blood iron levels through forced entry option. Analysis of variance (ANOVA) and t-test results assessed the degree of prediction of an outcome and whether a coefficient is different from 0, respectively. Bootstrapping was carried out to compute for robust estimates and confidence intervals in SLR. Predictors were then fitted into a full model using MLR, following the hierarchical method. Gestational age was entered into the first block using the forced entry option, while the other exploratory predictors were entered into the second block using the stepwise option. Data on daily consumption of iron supplements or multivitamins took the binary form of categorical variable (coded as 0 or 1). Estimates were used to define the best-fitting linear models, which take the form:

$$Y_i = (b_0 + b_n X_n) + \varepsilon_i \qquad (1)$$

Where Y_i is an outcome variable, X_n is a predictor variable, b_n is a parameter that quantifies the relationship between a predictor and outcome (*i.e.*, slope); b_0 is a parameter that tells the value of outcome when predictor is zero (*i.e.*, intercept of the line); and ε_i is the error associated with the prediction. In MLR, when multicollinearity or possible interactions between predictors were identified as reflected by unacceptable values of variance inflation factors, one of the variables found to have significant relationships in a pair was omitted from fitting into the full models.

Power was calculated using G*Power v.3.1.9.2. Where only one significant predictor was fitted in the model, effect size of 0.59 (*i.e.*, highest R^2 with TIBC as outcome) or 0.04 (*i.e.*, lowest R^2 with log_{10} SI as outcome) and a sample size of 109 achieved power values of 1.0 and 0.56, respectively. At the same time, fitting in six predictors in the full model resulted in power values ranging from 0.91 (for lowest R^2 obtained in Hct model) to 1.0 (for highest R^2 obtained in UIBC model). A p value less than 0.05 was considered statistically significant.

RESULTS

Profile of the Study Participants

Table 1 presents the socio-demographic characteristics, information on current pregnancy, and obstetric history of the study population (N=109). Pregnant women were 15–42 years of age, and majority (37%) were residing in *Brgy*. Panitian. The mean weight was 51.8 kg, but only 33% had desirable body weight according to gestational age. There was a high percentage of participants who were not working (81%), and most of them completed

Table 1. Socio-demographic characteristics and obstetric history of the study participants[a].

Parameters	N=109
Age (yr)	28.0 ± 7.0 (15–42)
Height (cm)	150.24 ± 5.17 (141.00–164.00)
Weight (kg)	51.83 ± 8.95 (35.55–75.10)
Within prescribed weight-for-height	36 (33.0)
Below prescribed weight-for-height	41 (37.6)
Above prescribed weight-for-height	32 (29.4)
BMI (kg/m^2)	22.91 ± 3.45 (16.38–32.08)
Area of Residence	
1st District/*Brgy.* Panitian	40 (36.7)
2nd District/*Brgy.* Alfonso XIII	28 (25.7)
3rd District/*Brgy.* Isugod	24 (22.0)
4th District/*Brgy.* Alfonso Bliss	17 (15.6)
Gestational Age (mo)	5.4 ± 1.9 (1–9)
Stage of Pregnancy	
First trimester	21 (19.3)
Second trimester	53 (48.6)
Third trimester	35 (32.1)
Gravidity	4.0 ± 2.4 (1–11)
Parity	3.0 ± 2.4 (0–9)
With Prenatal Check-ups[b]	74 (67.9)
With Daily Iron Supplement or Multivitamins	59 (54.1)
Estimated Monthly Household Income (Php)[b]	5,533.94 ± 5,178.57 (400.00–26,000.00)
Occupation[b]	
None	88 (80.7)
Full-time	12 (11.0)
Part-time	2 (1.8)
Others	4 (3.7)
Educational Level[b]	
No Education	8 (7.3)
Primary Education	25 (23.6)
Secondary Education	46 (42.2)
College Education	21 (19.2)
Vocational	2 (1.8)
Others	4 (3.7)

Notes:
[a]Age, height, weight, BMI, gestational age, gravidity, parity, and estimated monthly household income are presented as mean ± SD (range). Other data were presented as n (% based on N).
[b]With three missing observations

or had at least reached secondary education (42%). The mean estimated income per household per month was at Php 5,500.

The mean age of gestation of participants was at five months. Almost half were on their second trimester of pregnancy, while 19% were on their first trimester and 32% were on their last trimester. More than half of the pregnant women (68%) had prenatal check-ups with either a doctor, midwife, or nurse. A greater number of participants (54%) had consumed iron or multivitamin supplements daily such as ferrous sulfate, Usanatal, and Martham. Gravidity ranged from one to 11, from which 27 (25%) were pregnant for the first time (*i.e.*, primigravidae). Gravida 2, 3, and ≥4 were 19%, 13%, and 43%, respectively. Parity ranged from zero to nine. Pregnant women who had never given birth (*i.e.*, nulliparous) corresponded to 26% of the study participants.

The mean levels of the hematological and biochemical parameters that were used to assess the blood iron level of participants were within the prescribed normal ranges, except for TIBC (Table 2). Using the WHO Hb cut-off points, 38% of the pregnant women were found anemic. More than half of this group (22 or 54%) remained anemic despite daily consumption of oral iron supplements or multivitamins. Among the non-anemic participants, 37 or 54% were taking supplements daily. When the stage of pregnancy was considered (*i.e.*, CDC cut-offs), the percentage of anemic participants decreased to 28%. Meanwhile, the overall proportion of participants with depleted iron stores was 47% and 37% based on WHO and CDC cut-offs for SF, respectively. More than half of the anemic participants were iron-deficient (26 based on WHO cut-offs; 17 based on CDC cut-offs). The incidence of IDA was at 24% and 16%, respectively. Anemia without iron deficiency was at 14%, while the percentage of non-anemic participants who were iron-deficient was 23% (n=25) according to WHO cut-offs. In classifying the computed TSAT levels (*i.e.*, CDC cut-off), 21% of the participants were found iron-deficient). A high percentage of participants (79%) had normal SI levels, while only one-third (29%) had normal TIBC levels.

Simple Linear Models of Blood Iron Concentration and Status

Table 3 presents the association between each parameter and variables that may be used in predicting levels of blood iron, namely gestational age, body weight, gravidity, and parity. Pearson correlation coefficients show that gestational age and weight have significant positive association with TIBC and UIBC levels. The strength of association with weight was small or weak (r=0.1–0.3) while for gestational age, the strength of association was large or strong (r=0.5–1.0). On the other

Table 2. Hematological and biochemical characteristics of the study participants[a].

Parameters	*N*=109
Hb (g/dL)	11.18 ± 1.08 (8.6-14.6)
Anemic, WHO cut-off (<11 g/dL)	41 (37.6)
Mild (10.0-10.9 g/dL)	29 (26.6)
Moderate (7.0-9.9 g/dL)	12 (11.0)
Anemic, CDC cut-off[b]	31 (28.4)
Hct (%)	33.81 ± 3.33 (27-45)
Anemic, WHO cut-off (<33%)	40 (36.7)
Mild (30-32%)	34 (31.2)
Severe (<29%)	6 (5.5)
Anemic, CDC cut-off[c]	34 (31.2)
SF (ng/mL)	30.40 ± 32.37 (3-177)
Iron-deficient, WHO cut-off (<15 ng/mL)	51 (46.8)
Iron-deficient, CDC cut-off (<12 ng/mL)	40 (36.7)
SI (mg/L)	1.02 ± 0.41 (0.26-2.69)
Below reference range	13 (11.9)
Within reference range (0.6-1.6 mg/L)	86 (78.9)
Above reference range	10 (9.2)
TIBC (mg/L)	4.38 ± 1.04 (2.60-6.46)
Within reference range (2.51-3.63 mg/L)	32 (29.4)
Above reference range	77 (70.6)
UIBC (mg/L)	3.35 ± 1.12 (1.37-5.65)
TSAT (%)	24.80 ± 11.09 (5.84-49.87)
Iron-deficient, CDC cut-off (<16%)	23 (21.1)

[a]Blood iron levels are presented as mean ± S.D. (range). Frequencies of pregnant women who were anemic or iron-deficient and fell within, below, or above cut-off levels are presented as *n* (% based on *N*).
[b]Depends on stage of pregnancy: Anemia is Hb<11 g/dL for pregnant women on 1st and 3rd trimesters of pregnancy, and Hb<10.5 g/dL for pregnant women on 2nd trimester of pregnancy.
[c]Depends on stage of pregnancy: Anemia is Hct<33% for pregnant women on 1st and 3rd trimesters of pregnancy, and Hct<32% for pregnant women on 2nd trimester of pregnancy.

hand, the following significant negative associations were observed: (1) gestational age and Hb, Hct, SF, $\log_{10}$ SF, SI, $\log_{10}$ SI, and TSAT; (2) gravidity and $\log_{10}$ SF; and (3) parity and $\log_{10}$ SF. The strength of negative associations between gestational age and levels of SI, $\log_{10}$ SI, and Hct were small. There was also a small, negative association between gravidity or parity and $\log_{10}$ SF. Between gestational age and levels of Hb, SF, $\log_{10}$ SF, and TSAT, the degree of negative association was medium or moderate (r=0.3–0.5). The results of correlation between outcomes and maternal age, height, or estimated monthly household income were not presented as no significance was detected among variables.

Table 4 presents the SLR results using gestational age as a single predictor. Among the models presented, only the simple linear model for SI showed non-significance at an alpha level of 0.05. However, the use of $\log_{10}$ SI data resulted in a significant linear model. Thus, gestational age was shown to contribute significantly in estimating blood iron levels, and that the models predict the levels of iron biomarkers significantly well. An increase in gestational age by one unit will result in a decrease in Hb by 0.19 g/dL. On the other hand, a one unit-increase in gestational age will lead to an increase in TIBC by 0.32 mg/L. R^2 values ranging from as low as 0.04 up to 0.35 represent the variation in the parameters as explained by the models. Hence, 4% and 35% of the variations in $\log_{10}$ SI and UIBC, respectively, can be explained by the models. The remaining percentages of variation are attributed to other variables that also influence the outcomes. Other potential predictors tested, however, did not give significant results using SLR (data not presented).

Best-fitting Linear Models of Blood Iron Concentration and Status

The full models derived from a hierarchical MLR are presented in Table 5. The initial models for Hb, Hct, $\log_{10}$ SF, and UIBC refer to the first stage when only gestational age was evaluated as a predictor (*i.e.*, result of the forced entry method in the first block), while the second models present the best-fitting final models that included other significant predictors (*i.e.*, result of the stepwise method in the second block). The R values of the final models for Hb (0.42), Hct (0.39), and $\log_{10}$ SF (0.49) levels show moderate correlation with the significant predictors (*i.e.*, gestational age and weight for Hb and Hct; gestational age and parity for $\log_{10}$ SF). For UIBC level, the correlation with gestational age and daily consumption of iron or multivitamin supplements was strong (R=0.65). The variability in Hb, Hct, $\log_{10}$ SF, and UIBC levels of the participants that were accounted for by their individual predictors are 18%, 15%, 24%, and 42%, respectively.

The contribution of new predictors in explaining the variations in the outcome measures, aside from gestational age, can be assessed based on the change in R^2 values. Comparing the full models with the initial models indicates that weight accounts for an additional 5% and 4% of the variances in Hb and Hct levels of participants, respectively. Similarly, parity accounts for an additional 4% of the variance in $\log_{10}$ SF levels, while daily consumption of supplements contributes an additional 2% in the variability of UIBC levels. The final

Table 3. Association of potential predictors with levels of iron biomarkers of study participants.

Outcomes	Gestational age		Weight		Gravidity		Parity	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Hb (g/dL)	–0.337	<0.001*	0.125	0.098	–0.047	0.313	–0.048	0.310
Hct (%)	–0.294	0.001*	0.128	0.093	–0.078	0.210	–0.064	0.254
SF (ng/mL)[ab]	–0.425	<0.001*	–0.144	0.068	–0.133	0.084	–0.125	0.097
Log_{10} SF	–0.431	<0.001*	–0.143	0.070	–0.173	0.036*	–0.174	0.035*
SI (mg/L)[a]	–0.169	0.040*	–0.005	0.477	0.085	0.190	0.096	0.161
Log_{10} SI	–0.198	0.039*	–0.011	0.913	0.096	0.319	0.107	0.267
TIBC (mg/L)	0.574	<0.001*	0.183	0.029*	0.066	0.247	0.060	0.267
UIBC (mg/L)	0.594	<0.001*	0.172	0.037*	0.031	0.376	0.021	0.414
TSAT (%)	–0.482	<0.001*	–0.091	0.174	0.041	0.338	0.052	0.297

Notes:
[a]Data is positively skewed; hence, log_{10} transformation was applied.
[b]With detected outliers but were corrected upon log_{10} transformation
*Significant at $p < 0.05$ using Pearson correlation coefficient

Table 4. Linear models predicting levels of iron biomarkers with gestational age[a].

Outcomes	b_0	SE b_0	*p*	b_1	SE b_1	*p*	β	R^2
Hb (g/dL)	12.23 (11.43,12.96)	0.30	<0.001*	–0.19 (–0.31, –0.07)	0.05	<0.001*	–0.34	0.11
Hct (%)	36.61 (34.25,38.80)	0.93	<0.001*	–0.52 (–0.90, –0.16)	0.16	0.002*	–0.29	0.09
SF (ng/mL)	69.78 (47.91,94.24)	8.59	<0.001*	–7.29 (–11.62, –3.66)	1.50	<0.001*	–0.43	0.18
Log_{10} SF	1.81 (1.57,2.06)	0.11	<0.001*	–0.10 (–0.14, –0.07)	0.02	<0.001*	–0.43	0.19
SI (mg/L)[b]	1.22 (1.02,1.41)	0.12	<0.001*	–0.04	0.02	0.079	–0.17	0.03
Log_{10} SI	0.29 (0.24,0.34)	0.03	<0.001*	–0.01 (–0.02, –0.001)	0.01	0.039*	–0.20	0.04
TIBC (mg/L)	2.66 (2.20,3.11)	0.25	<0.001*	0.32 (0.25,0.40)	0.04	<0.001*	0.57	0.33
UIBC (mg/L)	1.44 (0.98,1.91)	0.27	<0.001*	0.35 (0.26,0.45)	0.05	<0.001*	0.59	0.35
TSAT (%)	40.13 (34.97,45.23)	2.85	<0.001*	–2.84 (–3.63, –2.00)	0.50	<0.001*	–0.48	0.23

Notes:
[a]95% confidence intervals obtained using bootstrapping are reported in parentheses.
[b]ANOVA and t-test results show non-significance.
*Significant at $p < 0.05$ using linear regression analysis

models present the following: (1) negative relationship between Hb, Hct, SF, log_{10} SF, log_{10} SI, and TSAT and gestational age; (2) negative relationship between log_{10} SF and parity; (3) positive relationship between Hb, Hct, SF, log_{10} SF, and TSAT and weight; (4) positive relationship between TIBC and UIBC and gestational age, and; (5) negative relationship between TIBC and UIBC and daily consumption of supplements of the participants.

Estimates of the final models for SF, log_{10} SI, TIBC, and TSAT levels have almost the same values with the SLR results presented in Table 4. Standard errors associated with the beta values were observed to be minimal except for SF level. No variables were entered into a model equation in predicting SI level. Height and gravidity were excluded from the exploratory predictors since high multicollinearity exist between weight and height, as well as parity and gravidity.

Table 5. Final linear models of predictors of iron biomarkers[a].

	Models	b (95% CI)	SE b	β	R^2	ΔR^2	p
Hemoglobin (g/dL)	Step 1						
	Constant	12.32 (11.72,12.93)	0.30		0.13		<0.001
	Gestational age	–0.21 (–0.31, –0.10)	0.05	–0.36			<0.001
	Step 2						
	Constant	11.07 (9.90,12.24)	0.59		0.18	0.05	<0.001
	Gestational age	–0.24 (–0.35, –0.13)	0.05	–0.42			<0.001
	Weight	0.03 (0.01,0.05)	0.01	0.23			0.016
Hematocrit (%)	Step 1						
	Constant	37.07 (35.22,38.92)	0.93		0.11		<0.001
	Gestational age	–0.58 (–0.90, –0.26)	0.16	–0.33			0.001
	Step 2						
	Constant	33.53 (29.92,37.14)	1.82		0.15	0.04	<0.001
	Gestational age	–0.67 (–1.00, –0.34)	0.16	–0.38			<0.001
	Weight	0.08 (0.01,0.15)	0.03	0.21			0.027
Serum Ferritin (ng/mL)	Constant	72.50 (55.12,89.88)	8.76		0.20	--	<0.001
	Gestational age	–7.61 (–10.63, –4.59)	1.52	–0.44			<0.001
Log_{10} Serum Ferritin	Step 1						
	Constant	1.84 (1.61,2.07)	0.12		0.20		<0.001
	Gestational age	–0.10 (–0.14, –0.06)	0.02	–0.45			<0.001
	Step 2						
	Constant	1.93 (1.69,2.18)	0.12		0.24	0.04	<0.001
	Gestational age	–0.10 (–0.14, –0.07)	0.02	–0.45			<0.001
	Parity	–0.04 (–0.07, –0.003)	0.02	–0.19			0.031
Log_{10} Serum Iron	Constant	0.29 (0.24,0.35)	0.03		0.04	--	<0.001
	Gestational age	–0.01 (–0.02, –0.001)	0.01	–0.20			0.037
Total Iron Binding Capacity (mg/L)	Constant	2.56 (2.07,3.04)	0.25		0.37	--	<0.001
	Gestational age	0.33 (0.25,0.42)	0.04	0.61			<0.001
Unsaturated Iron Binding Capacity (mg/L)	Step 1						
	Constant	1.33 (0.82,1.84)	0.26		0.40		<0.001
	Gestational age	0.37 (0.28,0.46)	0.05	0.63			<0.001
	Step 2						
	Constant	1.31 (0.80,1.81)	0.25		0.42	0.02	<0.001
	Gestational age	0.41 (0.31,0.51)	0.05	0.70			<0.001
	Iron supplement[b]	–0.37 (–0.74, –0.003)	0.19	–0.17			0.048
Transferrin Saturation (%)	Constant	40.66 (35.00,46.32)	2.86		0.25	--	<0.001
	Gestational age	–2.89 (–3.87, –1.90)	0.50	–0.50			<0.001

Notes:
[a]Analyzed using hierarchical multiple regression analysis: Gestational age was entered in the first block using forced entry method, while the rest of potential predictors (*i.e.*, maternal age, weight, parity, estimated monthly household income, and consumption of daily iron supplements or multivitamins) were entered into the second block using stepwise method.
[b]Coded as 0 for no and 1 for yes

DISCUSSION

Pregnant women are at risk of developing anemia due to iron deficiency because of elevated iron demands throughout pregnancy. If not properly addressed, anemia in pregnancy will increase the risk of maternal and perinatal mortality. This necessitates the identification of factors that significantly contribute to blood iron concentration and status during pregnancy.

Gestational Age and Weight are Predictors of Hb and Hct

Hb and Hct are the recommended and most commonly used parameters in determining anemia in the general population (WHO 2017a). Hb measures the level of the iron-rich hemoglobin protein in RBCs that transfer oxygen to other tissues in the body, while Hct determines the volume percentage of RBCs in whole blood (Billett 1990). The present study shows that gestational age and body weight were significant predictors of both Hb and Hct. Gestational age was a moderate, negative predictor of both parameters – lowering Hb by 0.24 g/dL and Hct by 0.67% per every increase in month of pregnancy while controlling for weight. On the other hand, weight increases Hb by 0.03 g/dL and Hct by 0.08% while holding gestational age constant. The decrease in Hb and Hct levels that was contributed by gestational age outweighs the almost negligible increase brought about by body weight.

The negative association between gestational age and Hb concentration in this study replicated previous findings in groups of pregnant women from Ethiopia (Melku *et al.* 2014) and Nigeria (Onoh *et al.* 2015). Anemia, as assessed by Hb level, was increasing and found to be higher toward the third trimester of pregnancy. In terms of weight as a positive predictor of Hb in the present study, Bodnar and colleagues (2004) have alternatively showed that inadequate weight gain was one of the negative predictors of Hb in a group of low-income pregnant women from North Carolina. Being underweight was also one of the significant predictors of Hb in Ethiopian pregnant women (Melku *et al.* 2014). Aside from providing further evidence that gestational age and weight were significant predictors of Hb during pregnancy, the present study was the first to report that gestational age and weight were significantly contributing to Hct in pregnant Filipino women.

It is worthy to note that the incidence of anemia among pregnant Filipino women in this study was 37.6% based on the WHO cut-off criterion for Hb. This was higher as compared to pregnant women with anemia from Ethiopia (16.6%), Zimbabwe (22.0%), and Uganda (32.5%) (Melku *et al.* 2014; Friis *et al.* 2001; Ononge *et al.* 2014). In fact, the most recent nationwide prevalence of anemia among pregnant Filipino women (24.6%) is still considered moderately high despite its continuous decline over the last NNSs. This stresses the need to come up with sustainable public health interventions and policy actions that will improve Hb and Hct concentrations as weeks or months of gestation progress during pregnancy.

Gestational Age and Parity are Predictors of SF

SF test measures an individual's iron stores. Depleted iron stores normally indicate iron deficiency, while high levels of iron stores suggest hemochromatosis or iron overload in the body (WHO 2011b). Gestational age and parity were both identified as negative predictors of SF concentration ($\log_{10}$-transformed) in the present study. The regression coefficient of gestational age was –0.10, which corresponded to a $10^{-0.10}$ or 0.79 times lower SF in ng/mL per unit increase in month of pregnancy while controlling for parity. Meanwhile, the coefficient of parity corresponded to $10^{-0.04}$ or 0.91 lower SF per unit increase in the number of livebirths and stillbirths while controlling for gestational age.

Results of the present study are in agreement with the findings of Friis and colleagues (2001), which involved a group of Zimbabwean pregnant women. The regression coefficients at gestational ages 25–28, 28–32, and 32–35 weeks were 0.84, 0.76, and 0.71, respectively. Hence, SF was lower in weeks 32–35 (*i.e.*, approximately 8th month of pregnancy) than in weeks 22–25 (*i.e.*, 6th month). A multi-center study participated by around 2,000 pregnant women in the United Kingdom also presented that increasing parity was one of the significant predictors of having anemia as reflected by Hb and SF concentrations (Barroso *et al.* 2011). Parity was also significantly associated with the likelihood of being anemic among pregnant women in Uganda (Onong*e et al.* 2014), although only Hb level was measured. Multiple pregnancies lead to iron depletion in a woman's body, most especially if prior pregnancies are closely spaced. Hence, the observed decline in SF concentrations as parity increases.

Gestational Age and Daily Consumption of Iron or Multivitamin Supplements are Predictors of UIBC

SI, TIBC, and TSAT are three parameters that assess the amount of iron in plasma or serum (Beard 2007). SI test measures the amount of total iron content per unit volume of blood, TIBC test measures the number of binding sites for iron atoms on transferrin (*i.e.*, amount of transferrin in the blood that is void of iron), and TSAT estimates the percentage of the two occupied binding sites on all transferrin proteins. UIBC is derived from SI and TIBC and reflects the considerable extra iron-binding capacity. Reduced SI in combination with increased TIBC confirm iron deficiency. Furthermore, UIBC is elevated but TSAT is reduced in case of iron deficiency (WHO 2017a).

In the present study, gestational age was positively associated with TIBC and UIBC but negatively contributing to $\log_{10}$ SI and TSAT of the participants. The overall effect was worsening of iron status as gestational age progresses. In a study participated by rural Thai-Muslim pregnant women (Piammongkol *et al.* 2006), gestational age at 34 weeks (*i.e.*, 7.5 months of pregnancy) resulted in a negative coefficient. This suggested an increased risk for iron deficiency and IDA as reflected by Hb, Hct, SF, SI, TIBC, and TSAT indices using an ordinal logistic regression model. On the other hand, daily consumption of iron or multivitamin supplements was shown as a significant negative predictor of UIBC in this study, with a magnitude almost comparable to but with opposite effect as gestational age (*i.e.*, iron supplementation lowered UIBC by 0.37 mg/L while gestational age increased UIBC by 0.41 mg/L). Given that only half of the participants were taking iron or multivitamin supplements daily, additional measures that will encourage and engage pregnant Filipino women to consume prenatal iron supplements daily and immediately after conception should be undertaken. Aside from strict monitoring throughout pregnancy, the provision and free access to prenatal iron supplements should be considered mandatory, most especially to pregnant women residing in rural areas of the country. This strategy will compensate for functional iron deficiency during later stages of pregnancy.

The risk factors or the variables contributing to the decline in iron status during pregnancy that were investigated in this study require intervention to eliminate them. Other studies, however, identified additional significant predictors of blood iron concentration and status during pregnancy. HIV infection contributed to lower Hb of pregnant women from Zimbabwe (Friis *et al.* 2001), Uganda (Ononge *et al.* 2014), Ethiopia (Melku *et al.* 2014), and Nigeria (Onoh *et al.* 2015). A number of pregnant women in the Philippines were afflicted with HIV according to UNAIDS (2016); this may be considered an exploratory predictor of iron status in future research works. In addition, an association with decreased Hb (Ononge *et al.* 2014) and SF concentrations (Friis *et al.* 2001) were observed in countries where malaria is endemic such as those in the African continent. Palawan, where participants of this study came from, is a province in the Philippines where malaria infection is endemic (WHO 2017b). The effect of malaria parasitemia on anemia during pregnancy may be determined in the future as this will also reinforce malaria prevention strategies in the country. As of this writing, however, comparing anemia incidence rates between municipalities endemic for malaria versus non-endemic regions in the country is not feasible. Although data were provided in the 8th NNS (FNRI 2015), high coefficients of variation (i.e., CV>10%) due to small sample size were observed, which suggests that prevalence rates may not be precise. Moreover, no study has been conducted which assessed the predictors of blood iron status among pregnant women residing in a malaria-free province in the country. Assessing micronutrients as predictors of blood iron levels may also be explored as vitamin A, riboflavin, vitamin B12, folate, and zinc also affect erythropoiesis; any deficiency related to these may impair the iron status of a pregnant woman and her infant (Friis *et al.* 2001). Lastly, establishing the underlying genetic factors of anemia and iron deficiency in the Filipino population and integrating these inherent factors into regression models will result in a more comprehensive prediction of blood iron concentration and status. A study that determined the differences in iron status based on single nucleotide polymorphisms in *TMPRSS6* and *TF* genes (Timoteo *et al.* 2018) offered information on the potential genetic predictors of Hb, Hct, SF, SI, TIBC, UIBC, and TSAT.

This study had a cross-sectional design, and the findings depended on the data obtained from the study participants during the period of data collection. Hence, no causal inferences can be made from the data. Gestational age, which was determined as the main predictor of the different iron parameters evaluated, was measured in months. Some previous similar reports used gestational age in weeks or trimester of pregnancy. This may more accurately represent the dramatic change in Hb concentrations and anemia prevalence during pregnancy in a curvilinear fashion (WHO 2011a) and gestational age may be entered as a quadratic term in linear models. However, given the relationship observed between gestational age in months and other outcome variables in this study, linear model was chosen as it is more appropriate and suitable for the data. The study was also limited with relatively smaller sample size as compared to the studies mentioned in this paper. In effect, binomial logistic regression was not used to predict the odds of being anemic or iron-deficient during pregnancy from the variables investigated. It is recommended to investigate in the future whether gestational age or parity can predict anemia and iron deficiency in pregnant Filipino women, as well as if weight and daily consumption of iron or multivitamin supplements can predict normal blood iron levels. Most importantly, validation of the current research findings in a population of pregnant women coming from a malaria-free province or region in the country is highly warranted as this will further assess whether the models and significant predictors presented can be applied to pregnant Filipino women in general.

CONCLUSION

This study presents that gestational age is a significant predictor of Hb, Hct, SF, SI, TIBC, UIBC, and TSAT in a group of pregnant women residing in Quezon, Palawan. Other significant predictors were body weight, parity, and daily consumption of iron or multivitamin supplements. A unit increase in body weight increased Hb and Hct while controlling for gestational age. Similarly, daily consumption of supplements decreased UIBC. On the other hand, a unit increase in parity decreased $\log_{10}$ SF while holding gestational age constant. These findings help to target high-risk pregnant Filipino women and, at the same time, emphasize the need for more intensive, assertive, and sustainable public health measures that will combat anemia during pregnancy in the country.

ACKNOWLEDGMENT

The authors acknowledge the entire program team of “S&T Based Solutions towards Sustainable Strategy for Child Malnutrition: The First 1000 Days Window of Opportunity;” the local health and nutrition officers in Quezon, Palawan; and the participants of the study. The authors are indebted to Engr. Rosemarie G. Garcia and Mr. Edward Vincent J. Magtibay of the Nutrition Research and Development Group, DOST-FNRI for the technical review of the paper and guidance on the statistical analyses employed in the study.

Funding: The study was supported by the General Fund of DOST-FNRI and Grants-in-Aid Funds of the Philippine Council for Industry, Energy, and Emerging Technology Research and Development (DOST-PCIEERD).

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