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Hemoglobin Z-Scores to Assess Iron-Therapy during Pregnancy: Learnings from a Pragmatical Clinical Trial

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Key Messages

- Measurements of blood concentration of haemoglobin (Hb) are widely used to diagnose and follow maternal anaemia, particularly in low-income settings, but during pregnancy, Hb suffers physiologic changes which reduce its reliability.
- The values of Hb must be adjusted for its physiologic U-shaped curve throughout the weeks of pregnancy, which can be done by transforming into Z-scores (units of standard deviation from a reference mean).
- We advise to adopt *nomograms of Hb Z-scores* as an easy-to-use tool in managing anemia during the prenatal care, whilst health practitioners waiting for innovations on diagnostic tests for maternal anemia.

Keywords: Anemia; Iron-Deficiency; Pregnancy; Ferrous Compounds; Treatment Outcome; Hemoglobin

Abstract

Background: Iron-deficiency anemia is the commonest nutritional deficiency in pregnant women, especially in low- and middle-income countries. Simple parameters of red blood cells (e.g. blood hemoglobin concentration-Hb) are usually applied as proxy of iron-deficiency anemia. However, during pregnancy, they are biased by the physiological hemodilution.

Objective: To estimate the therapeutic dose-response of iron pills on the Hb values, considering the hemodilution phenomenon in pregnant women.

Methods: 144 anemic women (Hb < 11.0 g/dL), in the 2nd or 3rd trimesters of pregnancy, completed a single arm trial with two daily pills of 40 mg elemental iron (23 to 105 days of treatment). The therapeutic dose-response was estimated by post-pre-treatment differences in the Hb absolute values, as well in Z-scores (units of standard deviation [SD] from a Hb reference mean for the pregnancy week), via linear regression models. The independent variable was the number of pills ingested by each woman, and the adjusting confounders were socio-demographic and clinical characteristics, and treatment duration.

Results: We found no effects of the iron pills on Hb when it was measured by post-pre-treatment differences of absolute values. However, there was a linear rising in the post-pre-treatment differences of Hb Z-scores per pill ingested, particularly in women who were not previously in iron supplementation: 0.12 SD (± .004 SD; $p < 0.01$).

Conclusions: Hb Z-scores can be more reliable than Hb absolute values to evaluate the hematologic effect of iron-therapy during pregnancy. Population-specific nomograms of Hb Z-scores should be built and validated as an easy-to-use tool to manage maternal anemia.

Trial registration: Brazilian Clinical Trials Registry (RBR-237wbg) and World Health Organization International Clinical Trials Registry Platform (U1111-1123-2605).

Introduction

The global prevalence of anemia and iron deficiency are around 40% [**1,2]** and 20% **[3]**, respectively. Others nutritional deficiencies, inherited disorders and infections contribute for the global burden of anemia **[2]**, which can difficult the diagnosis of iron-deficiency anemia and may limit the success of iron supplementation programs. Although a low blood hemoglobin concentration (Hb) is a general parameter of anemia, it is widely used as proxy of iron-deficiency anemia (IDA) **[4]**, particularly in low- and middle-income countries (LMIC), because of its easy availability and low cost **[5]**. However, during pregnancy, the Hb is biased by a physiologic hemodilution, leading some authors to advice adjustments in Hb values per gestational week **[6, 7].**

During the first two trimesters of pregnancy, the plasma volume progressively increases up to 50%, while the red blood cells rise by 25% **[4, 8]**. This phenomenon causes a dilutional drop in Hb values, across the 2nd and 3rd trimesters of pregnancy, of around 1.4 g/dL (or 11% from the 1st trimester values) **[4]**. At the 3rd trimester of pregnancy, there is a gradual hemoconcentration until delivery, when the Hb can return to the baseline values [6, 8]. This dynamic oscillation of Hb values throughout pregnancy looks like a U-shaped curve, and it may induce one to incorrectly interpret it as changes in the body iron status **[6]**. This misinterpretation can bias the diagnostic of IDA and the assessment of therapeutic response to iron replacement in pregnant women **[6, 7]**.

Metanalyses of randomized clinical trials estimate increases of 0.4 g/dL on maternal Hb values after some weeks of daily treatments with 60 mg of elemental iron [9,10], and around 0.8 g/dL with iron doses of 60-90 mg **[9, 11]**. These dose-response effects were calculated in relation to control groups using placebo or iron-free supplements. These control strategy works to adjust the therapeutic effect for physiological changes, but it is not applicable to real prenatal settings. Given that, Beaton and McCabe had purposed converting an observed value of Hb into units of standard deviations (SD) from an expected mean (Z-scores) for the respective gestational week of a reference external population of healthy pregnant women **[6]**. These authors estimated by pooled analysis that daily iron-therapy during pregnancy results in a final Hb 0.29 SD higher than weekly, which means a difference of 0.26 g/dL in the Hb values **[6]**.

A correct and timely diagnosis and management of maternal IDA are critical, as a successful treatment can prevent prematurity and birthweight **[10, 11, 12]**. Moreover, deficits or excess of iron during pregnancy may injures the mental and psychomotor development of child **[13, 14]**. Iron biomarkers testing (ferritin, transferrin saturation, soluble transferrin receptor and hepcidin) are still not standardized for pregnant women **[4]**. So, the Hb Z-scores method may be suitable for adjusting the Hb according to the gestational week, and so surpass the hemodilution bias over the Hb assessment throughout pregnancy. From this point of view, this paper aims to show how different can be the therapeutic response to iron pills when it is estimated either by Hb absolute values or Z-scores in anemic pregnant women in a single prenatal-care center.

Methods

We conducted a pragmatic clinical trial to assess the hematologic responsiveness to a therapeutic test with oral iron. The study protocol **[15]** and the primary results were published elsewhere **[16]**. This current paper brings a posteriori data analysis, which was performed to stand the Hb Z-scores rather than absolute values for assessing IDA in pregnant women. Our objective was to estimate a dose-response effect of iron pills on the Hb values.

This study followed the ethics principles in research with human beings of the World Medical Association's Declaration of Helsinki and was approved by the Research Ethics Committee of Instituto de Medicina Integral Prof Fernando Figueira-IMIP (registration number 2050/10). Each participant was informed about the study objectives and asked to read and sign the consent form prior to their inclusion in the study. The protocol was registered in the International Clinical Trials Registry Platform (U1111-1123 -2605) of World Health Organization (WHO) and the Brazilian Registry of Clinical Trials of the Brazilian Ministry of Health.

Study Population, Setting, Period and Data

Women assisted at the pre-natal care outpatient at a public health facility in Recife (a city located at sea level in the Northeast of Brazil) were included if they were between 18 and 35 years old, in the 12th to 32nd weeks of low-risk singleton pregnancy and pre-classified as anemic according to the WHO's criteria (Hb <11.0 g/dL) **[5]**. The women were excluded if they had Hb ≤ 7.0 g/dL, history of hypersensitivity or intolerance to ferrous sulfate, mental disorders, and user of tobacco, alcohol or other drugs, or a diagnosis of another cause of anemia or infection.

Information on socio-economic characteristics (age, self-assigned ethnicity, educational level, family income per capita) and clinical-obstetric (BMI, previous pregnancies, weeks of pregnancy and use of oral iron before the study) were obtained at the time of the enrollment, afterwards the women were forwarded to blood collection to determine the clinical-laboratory pre-treatment variables: Hb, white blood cells count and serum ferritin. The Hb and white blood cells were analyzed using the flow cytometry and absorbance of the

automated hematology analyzer ABX Pentra DF120 manufactured by Horiba® and was expressed in g/dL end cell/mm^3, respectively. The serum ferritin levels were expressed in ng/mL and were obtained using the chemiluminescence method with ADVIA equipment – Centaur Ferritin, manufactured by Bayer®.

Intervention, Follow-Up, and Withdrawal Criteria

The treatment consisted of two daily doses of ferrous sulfate 109 mg pills with 40 mg of elemental iron (Hematofer®, Prati Donaduzzi & Cia LTDA). Three blisters with 20 pills were given at the enrollment (C_0) and at the next monthly revaluations (C_1, C_2). Pregnant women were oriented at each consultation to ingest the medication with a glass of drinking water, 30 minutes before a meal, and to preserve the non-consumed pills in the blisters. The women returned the pills not taken in the blisters for calculating the pills taken at C_1, C_2 and C_3.

The follow-up period was stopped earlier in case of genital bleeding, childbirth delivery, evolution to high-risk pregnancy, drop out of treatment, use of another type of iron supplement, cure (Hb ≥ 11.0 g/dL) or worsening of anemia (Hb < 7.0 g/dL or drop in Hb > 1.0 g/dL). These pregnant women were referred to an individualized conduct.

The dose-response effect of iron pills was measured by the differences between initial and final Hb values (post-pretreatment differences) using either the Hb absolute values (g/dL) or Hb Z-scores (SD units). The final Hb was obtained at the end of follow-up, ensuring the blindness of the participants, researchers, and health professionals. The Hb values of each woman were transformed a posteriori into Z-scores following the mathematical formula: Hb Z-score = (Hb value observed – mean of Hb expected)/0.9 (SD in the reference population) **[6]**.

The Z-scores quantified the difference in SD between the observed Hb value and the mean expected for the gestational week at the time of the blood sampling, according to a reference distribution curve of Hb means. Due to the lack of Brazilian population data, we used the Hb U-shaped curve reported by the Centers for Disease Control and Prevention **[18]**, which was also used by Beaton and McCabe to develop the Hb Z-score method **[6]**.

Data Analysis

The dose-response effect of iron pills on the Hb was estimated in two linear regression models with the software Stata/SE 12.1. The number of iron pills ingested by the women was applied in both models as the independent variable, and the Hb post-pretreatment differences were the dependent variable: in absolute values **(model 1)** or Z-scores **(model 2)**.

Bivariate and multivariate models were adjusted for the days of treatment. Multivariate models were also adjusted for socioeconomic and clinical-obstetric co-variables at the baseline. We adopted saturated models to calculate the mean dose-response effect of iron pills (β coefficient of SD per pill) adjusted for all confounders. Additionally, to examine an effect modification, we stratified the model 2 according to the use of iron supplementation before the study **(model 3)**, as well Atalah classification **(model 4) [17]**. Moreover, we introduced the interaction term 'ironpills·ferritin' in the model 3 to verify if the body iron status baseline could boost the dose-response effect of iron pills **(model 5)**. In all models, categoric variables were handled as dummy.

Variables	Hb Mean Difference (SD)		
	Crude Coef.	Adjusted Coef. (social and obstetric variables)	Adjusted Coef. (nutritional variables)
Iron pills	.003 (.003)	.004 (.003)	.005 (.003)
Days of treatment	-.003 (.005)	-.004 (.005)	-.007 (.005)
Socio-Economic Characteristics			
Self-assigned ethnicity Others		Ref.	Ref.
White		-.080 (.250)	.040 (.279)
Mixed		.078 (.232)	.105 (.258)
Black		-.044 (.248)	.016 (.267)
Educational level Incomplete elementary/high school or less		Ref.	Ref.

Complete elementary/high school or more		-.222 (.207)	-.236 (.183)
Family income *per capita* (R$)		-.000 (.000)	.000 (.000)
Clinical Characteristics			
Previous pregnancies Yes		Ref.	Ref.
No		-.182 (.206)	-.005 (.192)
Months since the last delivery		-.003 (.002)	-.000 (.002)
Weeks of pregnancy		.071 (.024)[c]	.051 (.024)
Gestational trimester Third		Ref.	Ref.
Second		.688 (.203)[c]	.524 (.198)
First		.145 (.480)	.218 (.456)
BMI classification[d]: underweight < adequate weight < overweight/obesity			.063 (.070)
Using oral iron before the study No			Ref.
Yes			-.176 (.116)
Laboratory results at the baseline			
Haemoglobin concentration (g/dL)			-.441 (.105)
Serum ferritin (ng/mL)			-.002 (.001)
White blood cells count (cell/mm^3)			$7.49 \cdot 10^{-6}$ (.000)
Constant		.403 (-1.254)	3.633 (3.636)
R^2	.007	0.172	0.332
N. Obs.	137	137	136[e]

R$: current Brazilian currency | SD: Standard Deviation | 95% CI: Confidence Interval of 95% | BMI: Body Mass Index | [a]p value < .10; [b]p value < .05; [c]p value < .01 | [d]Atalah *et al* 1997 | [e]A women had no data on income and BMI.

Table 1: Model 1- Regression analysis of dose-response effect of each iron pill on post-pretreatment differences of Hb absolute values; 137 Brazilian pregnant women, 2012.

Variables	**Hb Z-score Mean Difference (SD)**		
	Crude Coef.	Adjusted Coef. (social and obstetric variables)	Adjusted Coef. (nutritional variables)
Iron pills	.008 (.003)[b]	.007 (.003)[b]	.008 (.003)[c]
Days of treatment	-.014 (.006)[c]	-.014 (.006)[b]	-.018 (.006)[c]
Socio-Economic Characteristics			
Self-assigned ethnicity Others		Ref.	Ref.

White		-.051 (.286)	-.120 (.304)
Mixed		.097 (.260)	.184 (.277)
Black		-.005 (.275)	.111 (.288)
Educational level Incomplete primary/middle school or less		Ref.	Ref.
Complete elementary/high school or more		-.222 (.224)	-.258 (.209)
Family income *per capita* (R$)		.000 (.000)	.000 (.000)
Clinical Characteristics			
Previous pregnancies Yes		Ref.	Ref.
No		-.096 (.234)	.091 (.224)
Months since the last delivery		-.002 (.002)	.001 (.003)
BMI classification[d]			
1 (underweight), 2 (adequate weight), 3 (overweight/obesity)			.055 (.078)
Using oral iron before the study No			Ref.
Yes			-.269 (.139)[a]
Laboratory results at the baseline			
Haemoglobin concentration (g/dL)			-.500 (.118)[c]
Serum ferritin (ng/mL)			.0003 (.001)
White blood cells count (cell/mm^3)			-.00002 (.000)
Constant		.164 (.158)	.417 (.410)
R^2	.059	.073	.230
N. Obs.	137	137	136[e]

R$: current Brazilian currency | SD: Standard Deviation | 95% CI: Confidence Interval of 95% | BMI: Body Mass Index | [a]p value < .10; [b]p value < .05; [c]p value < .01 | [d]Atalah *et al* 1997 | [e]A women had no data on income and BMI.

Table 2: Model 2- Regression analysis of dose-response effect of each iron pill on post-pretreatment differences of Hb Z-scores; 137 Brazilian pregnant women, 2012.

Variables	Not using iron supplements	Using iron less than a month	Using iron for at least one month
	Hb Z-score Mean Difference (SD)		
Iron pills	.012 (.004)[c]	.005 (.032)	-.015 (.012)
Adjusted for days of treatment	YES	YES	YES
Adjusted for Socio-Economic Characteristics	YES	YES	YES
Adjusted for Clinical Characteristics	YES	YES	YES
R^2	.281	.538	.648
N. Obs.	97	18	22
[a]p value < .10; [b]p value < .05; [c]p value < .01			

Table 3: Model 3- Regression analysis of dose-response effect of each oral iron pill on post-pretreatment differences of Hb Z-scores, stratified according to the use of iron supplementation before the enrollment in the study; 137 Brazilian pregnant women, 2012.

Variables	Underweight	Adequate weight	Overweight/Obesity
	Hb Z-score Mean Difference (SD)		
Iron pills	.022 (.009)[b]	.012 (.006)[a]	-.002 (.006)
Adjusted for days of treatment	YES	YES	YES
Adjusted for Socio-Economic Characteristics	YES	YES	YES
Adjusted for Clinical Characteristics	YES	YES	YES
R^2	.674	.239	.483
N. Obs.	29	71	37
BMI: Body Mass Index | [a]p value < .10; [b]p value < .05; [c]p value < .01			

Table 4: Model 4- Regression analysis of dose-response of each iron pill on post-pretreatment differences of Hb Z-scores, stratified according to the Atalah classification for BMI of pregnant women at the baseline; 137 Brazilian pregnant women, 2012.

Variables	Overall sample	Not using iron before	Using iron (less than a month)	Using iron (at least a month)
	Hb Z-score Mean Difference (SD)			
Iron pills	.011 (.003)[c]	.015 (.004)[c]	-.017 (.056)	-.044 (.021)[a]
Serum ferritin (ng/mL)	.005 (.002)	.008 (.002)[b]	-.034 (.098)	-.025 (.025)
Iron pills x Ferritin (interaction term)	$-.51\cdot10^{-4}$ (.000)[b]	$-.74\cdot10^{-4}$ (.000)[b]	$.42\cdot10^{-3}$ (.001)	$-.39\cdot10^{-3}$ (.000)
Adjusted for days under treatment	YES	YES	YES	YES

Adjusted for Socio-Economic Characteristics	YES	YES	YES	YES
Adjusted for Clinical Characteristics	YES	YES	YES	YES
R^2	.263	.324	.561	.745
N. Obs.	137	97	18	22
[a]p value < .10; [b]p value < .05; [c]p value < .01				

Table 5: Model 5- Regression analysis showing the interaction effect of serum ferritin levels at the baseline with the dose-response effect of an iron pill (post-pretreatment differences of Hb Z-scores), according to the use of iron supplementation before the enrollment; 137 Brazilian pregnant women, 2012.

Results

From August 2011 to October 2012, 187 pregnant women were consecutively enrolled and, until December 2012, 144 were monthly followed by 23 to 105 days: 23 to 60 days in 54% (73/136) and 61 to 105 days in 46% (63/136). The women with full data (n 137) had a mean age of 25.8 years old (SD 4.8) and mean gestational age of 23 weeks (SD 5); 65% had concluded high school or superior level; 21% were underweight and 26% were overweight or obesity. The baseline mean of Hb was 10.3 g/dL (SD 0.65) and the median of serum ferritin was 24.6 ng/mL (interquartile 25-75% = 11-49 ng/mL). A conditional mean differences regression model (Supplementary Appendix) showed that the socio-economic and clinical characteristics at baseline were quite similar between women who ingested at least 100 pills of iron (n 57) and less (n 79), which means that, in our study sample, the number of pills ingested by the women was not driven by the co-variates.

(Tables 1 and 2) show the regression analyses estimating the effects of each iron pill ingested on the post-pretreatment differences in Hb absolute values (model 1) and Hb Z-scores (model 2). The coefficients are adjusted for the days under treatment and for the effect of social and obstetrics co-variables, and then for nutritional variables. We found no differences on Hb absolute values per pill ingested, but we found a positive and significant dose-response effect of iron pills on Hb Z-scores. The mean increment per pill on the maternal Hb Z-score was by 0.008 SD, independently of treatment days. This effect remained unchanged after inserting social, obstetric and nutritional confounders in the model. Eventually, there were significant negative effects on the post-pretreatment differences in Hb Z-score by the baseline Hb of the women (-0.5 SD per 1.0 g/dL of Hb) and by the duration of treatment (-0.018 SD per day).

(Tables 3 and 4) show the regression models stratified according to the use of iron supplements before the enrollment at the study (model 3) and the BMI classification at the baseline (model 4). In both, we found an effect modification on the dose-response effect of iron pills, as it remained significant only in women who were naïve of iron supplements before entry in the trial (0.012 SD per iron pill) and in women with underweight (0.022 SD per iron pill). In the model 5 (Table 5) we examined the interrelation between the use of iron supplements before the enrollment, the baseline serum ferritin and the dose-response effect of iron pills, by adding to model 3 an interaction term between serum ferritin and the number of pills ingested during the trial. This model showed that the dose-response among pregnant women who were iron-naïve was reduced by 0.000074 Hb Z-score per iron pill for each ng/mL of maternal ferritin at the baseline.

Discussion

Our analysis failed to show a dose-response effect of a pill of ferrous sulfate (40 mg of iron) on the maternal Hb absolute values, after 23 to 105 days of daily iron-therapy in anemic pregnant women. On the other hand, it was found a robust mean increase of 0.008 Hb Z-score per iron pill, independently of treatment duration. This effect was significant and become higher in underweight women (0.012 Hb Z-score per iron pill) and in iron-naïve women (0.022 Hb Z-score per iron pill). These findings mean that after 30 iron pills the maternal Hb would rise around 0.36 Z-score in underweight women, and 0.66 Z-score in iron-naïve women. Particularly in iron-naïve women, the body iron status at the baseline negatively interacted with the dose-response effect of iron pills at a coefficient of -0.000074, which means: the higher baseline ferritin level, the lower Hb Z-score increase per pill.

Dose-Response Effect of Iron on Hb Absolute Values

Nowadays a set of evidence about the hematologic effect of iron-therapy in pregnant women is based on the assessment of changes in Hb values at a group level, regarding the total posology prescribed after long term treatments **[9, 11, 19]**, rather than estimates of single dose-responses. A lack of study data on iron doses ingested by the women precludes

estimating precise correlations between the iron doses and the hematologic effect. Kehoe et al observed that a third of the randomized trials with nutritional supplements in pregnant women failed to describe how therapeutic compliance was assessed and a half failed to report it numerically **[20]**.

This issue has been dealt in few papers. Ekström et al applied a pill bottle equipped with an electronic counting device to evaluate the adherence to bird-day pills with 60 mg of elemental iron. They estimated a final effect of 0.058 g/dL on maternal Hb per 100 mg of iron ingested **[21]**. Then, these same authors conducted a multicenter study using the same technology to assess the therapeutic adherence in 90 anemic pregnant women, and they found a final effect of 0.034 g/dL on maternal Hb per pill ingested with 60 mg of iron **[22]**. In turn, Roberfroid et al used the counting of pills ingested by 600 anemic pregnant women and found an effect of 0.006 g/dL per pill with 30 or 60 mg of iron **[23]**.

In our study the counting of pills along with an array of treatment duration resulted in a broad range of iron doses intake around a mean of 90 pills ingested. However, there were no significant dose-response effect on Hb absolute values, disagreeing with those previous studies **[21, 22, 23]**. This result may be explained by the U-shaped curve of Hb during pregnancy. In our study, the participant follow-up starting around 23rd gestational week, lasting 8 to 14 weeks. This gestational period comprises the nadir of Hb U-shaped curve (24-28 gestational weeks) **[6]**. So, the Hb absolute values may have fallen from the baseline level and then risen to similar levels, which can mask a therapeutic effect. This might mean that absolute values of Hb have no reliability to assess the therapeutic response to oral iron, particularly, when one cannot compare it in a control group, such as in a real health care setting.

Dose-Response Effect of Iron on Hb Z-Scores

Our analysis on Hb Z-scores disclosed a significant dose-response effect of a single iron dose. Accordingly, Beaton and McCabe reanalyzed 21 trials with oral iron in children and pregnant women and observed dissent results when therapeutic responses were assessed by Hb absolute values or Hb Z-scores, pointing towards the importance to distinguish between physiological and therapeutic effects **[6]**. Including the baseline Hb values or the gestational age in regression models, such was made by previous authors **[21,22,23]** and by ourselves (model 1), is not enough to deal with the complex inter-relation among gestational age, treatment length and Hb changes, as it has a curvilinear rather than a linear behavior **[6]**.

Therefore, to run the regression model 2, we converted the measured Hb values pre- and post-treatment into Z-scores to individually adjust it for gestational age at the beginning and the end of treatment, according to reference means of Hb at each gestational week **[6, 18]**. A Brazilian reference curve would be more suitable for our analysis, but there are no national data, and so CDC's curve worked as an international standard to normalize the intra-observation variabilities (Hb curve in each woman) and inter-observation (different gestational ages and treatment duration across the women) **[6, 7]**. Population-specific reference curves of maternal Hb tend to become a necessity at clinical and research field, since the current evidence indicates the benefit of iron supplementation on perinatal outcomes (prematurity and birthweight) **[10, 11, 12]**. Therefore, placebo groups in iron-therapy trials should no more advisable.

So far, the Z-score method has not been widely used. Among high quality studies comprised by the Cochrane's metanalysis on efficacy of maternal iron-therapy **[19]**, only Mumtaz et al converted the Hb values into Z-scores to evaluate the dose-response after 12 weeks of iron-therapies **[24]**. They found that the Hb improvements after 100 mg of oral iron twice a week were 0.015 Z-score bellow the improvements after daily posology **[24]**. In the same Cochrane's metanalysis, Souza et al also compared twice-weekly with daily iron-therapies, but these authors had not corrected the Hb values by Z-scores and found no differences between groups at 16th week of treatment with 60 mg of iron **[25]**. We have reanalyzed the Souza et al databank by transforming each measured maternal Hb into Z-scores. Eventually, we disclosed that the twice-weekly scheme improved the Hb 0.40 Z-score less than daily **[7]**, agreeing with results of Mumtaz et al trial **[24]**.

Our previous results **[7]**, along with our present findings, show how different may look the effect of iron-therapy during pregnancy if it is measured by changes on Hb absolute values or Hb Z-scores. Here we showed that the effect of a single iron pill may be observed by Hb Z-scores, even within a short term and low dose treatment. So, a precise and personal management of the oral iron-therapy in pregnant women is possible and urged, particularly in underweight or iron-naïve women, who have the nutritional iron-deficiency as the main cause of their anemia. On the other hand, women who are not underweight and already is taking iron-supplements may have other important causes of anemia, as infectious and hypervolemic conditions. In these women, a short therapeutic test with oral iron to confirm the maternal iron needs would be very useful **[26, 27]**. However, during pregnancy, a timely therapeutic test with oral iron seems to be reliable only if one take in account the physiologic Hb U-shaped curve.

Indeed, time is a critical issue to opportunely improve the iron status of women during pregnancy, as well to prevent iron excess in anemic women who are unresponsive to iron therapy. Give that, the Hb Z-scores method can allow one to early observe the effect of iron-therapy, without the time-dependent distortions of Hb U-shaped curve. This method is especially promising to evaluate the efficiency of programs for

anemia control and to assess the therapeutic efficacy of iron in the real prenatal care practice. For this purpose, nomograms of Hb Z-scores (Supplementary Appendix) may become a suitable and pragmatic graphic tool to guide the clinical decision-making on maternal anemia, as well as to improve the success of iron supplementation programs and policy.

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References

1. World Health Organization (2015) The global prevalence of anaemia in 2011. Geneva: WHO. Available from: https://www.who.int/publications/i/item/9789241564960
2. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Nutrition Impact Model Study Group (Anaemia), et al. (2013) Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995–2011: a systematic analysis of population-representative data. Lancet Global Health 1: 16-25.
3. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, et al. (2013) Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 382:427–451.
4. Benson CS, Shah A, Frise MC, Frise CJ (2021) Iron deficiency anaemia in pregnancy: a contemporary review. Obstet Med 14(2):67-76. doi:10.1177/1753495X20932426.
5. World Health Organization (2011) Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity - WHO Vitamin and Mineral Nutrition Information System (WHO/NMH/NHD/MNM/11.1). Geneva: WHO. Available from: http://www.who.int/vmnis/indicators/haemoglobin.pdf
6. Beaton GH, McCabe GP (1999) Efficacy of intermittent iron supplementation in the control of iron deficiency anaemia in developing countries - an analysis of experience. Final report to the Micronutrient Initiative. Toronto: Canadian International Development Agency.
7. Bresani-Salvi CC, Braga MC, Batista Filho M (2014) Diagnostic accuracy of hemoglobin for iron deficiency in pregnancy: disclosing results of a cited clinical trial. Pan-American Journal of Public Health 36: 110-116.
8. Cao C, O'Brien KO (2013) Pregnancy and iron homeostasis: an update. Nutrition Review 71:35-51.
9. Sloan NL, Jordan E, Winikoff B (2002) Effects of iron supplementation on maternal haematologic status in pregnancy. Am J Public Health 92: 288-293.
10. Haider B, Olofin I, Wang M, Spiegelman D, Ezzati M, et al. (2013) Anaemia, prenatal iron use, and risk of adverse pregnancy outcomes: systematic review and meta-analysis. British Medical Journal 346:f3443. doi: 10.1136/bmj.f3443
11. Peña-Rosas JP, De-Regil LM, Dowswell T, Viteri FE (2012) Daily oral iron supplementation during pregnancy. Cochrane Database of Systematic Review 12:CD004736. doi: 10.1002/14651858.CD004736.pub4
12. World Health Organization (2012) WHO guideline: daily iron and folic acid supplementation in pregnant women. Geneva: WHO. Available from: http://apps.who.int/iris/bitstream/10665/77770/1/9789241501996_eng.pdf
13. Iglesias L, Canals J, Arija V (2017) Effects of prenatal iron status on child neurodevelopment and behaviour: a systematic review. Crit Rev Food Sci Nutr 58: 1604-1614. doi: 10.1080/10408398.2016.1274285.
14. Janbek J, Sarki M, Specht IO, Heitmann BL (2019) A systematic literature review of the relation between iron status/anemia in pregnancy and offspring neurodevelopment. Eur J Clin Nutr 73: 1561-1578.
15. Bresani CC, Braga MC, Felisberto DF, Tavares-de-Melo CE, Salvi DB, et al. (2013) Accuracy of erythrogram and serum ferritin for the maternal anaemia diagnosis (AMA): a phase 3 diagnostic study on prediction of the therapeutic responsiveness to oral iron in pregnancy. BMC Pregnancy and Childbirth 16:13. doi: 10.1186/1471-2393-13-13
16. Bresani-Salvi CC, Braga MC, Figueirôa JN, Batista-Filho M (2016) Could the erythrocyte indices or serum ferritin predict the therapeutic response to a trial with oral iron during pregnancy? Results from the Accuracy study for Maternal Anaemia diagnosis (AMA). BMC Pregnancy and Childbirth 16: 218.
17. Atalah SE, Catillo LC, Castro S.R., Aldea P.A. (1997) Propuesta de un nuevo estándar de evaluación nutricional embarazadas. Revista Médica del Chile 125: 1429-1436.
18. Centers for Disease Control and Prevention (1998) Recommendations to prevent and control iron deficiency in the United States. MMWR Recomm Rep 47: 1-36.
19. Reveiz L, Gyte GML, Cuervo LG, Casasbuenas A (2011) Treatments for iron-deficiency anaemia in pregnancy. Cochrane Database of Systematic Review 10:CD003094. doi: 10.1002/14651858.CD003094.pub3
20. Kehoe S, Chheda PS, Sirazul S, Baird J, Fall CHD (2009)

Reporting of participant compliance in randomized controlled trials of nutrition supplements during pregnancy. Maternal and Child Nutrition 5: 97-103.

21. Ekström ECM, Kavishe FP, Habicht JP, Frongillo EAJr, Rasmussen KM, et al. (1996) Adherence to iron supplementation during pregnancy in Tanzania: determinants and haematologic consequences. American Journal of Clinical Nutrition 64: 368-374.
22. Ekström EC, Hyder SM, Chowdhury AM, Chowdhury SA, Lönnerdal B, et al. (2002) Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues. American Journal of Clinical Nutrition 76: 1392-1400.
23. Roberfroid D, Huybregts L, Habicht JP, Lanou H, Henry MC, et al. (2011) Randomized controlled trial of 2 prenatal iron supplements: is there a dose-response relation with maternal hemoglobin? American Journal of Clinical Nutrition 93:1012-1008.
24. Mumtaz Z, Shahab S, Butt N, Rab MA, DeMuynck A (2000) Daily iron supplementation is more effective than twice weekly iron supplementation in pregnant women in Pakistan in a randomized double-blind clinical trial. Journal of Nutrition 130: 2697-2702.
25. Souza AI, Batista-Filho M, Ferreira LOC, Figueirôa JN (2004) The effectiveness of three regimens using ferrous sulfate to treat anaemia in pregnant women. Panamerican Journal of Public Health 15: 313-319.
26. Pavord S, Myers B, Robinson S, Allard S, Strong J, et al. (2012) UK guidelines on the management of iron deficiency in pregnancy. British Journal of Haematology 156: 588-600.
27. World Health Organization (2001) Iron deficiency anaemia: assessment, prevention and control – a guide for programme managers. Geneva: WHO.

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