Iron profile and Hepcidin Associated with Oxidative Stress and Metabolic Disturbances in Pregnancy

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Abstract

Background: A common problem during pregnancy is anemia and to reduce its prevalence the WHO and national guidelines recommend a prescription of 30 to 60 mg of iron/day. The aim of this study was to evaluate the association of iron profile, hepcidin and oxidative stress in pregnant women prescribed with iron as a probable cause of metabolic disorders.

Method: In this cohort study two groups were followed: A) women with low-risk pregnancy (WLRP), B) women with high-risk pregnancy (WHRP): women with metabolic disorders (dyslipidemias, GDM and high blood pressure). Oxidative stress enzymes, iron profile and hepcidin were measured in the second and third trimesters.

Results: There were significant differences in hepcidin levels between WLRP and WHRP in 2nd (3.6 ± 4.2 vs 4.69 ± 3.23 P=0.005) and 3rd trimester (3.65 ± 3.44 vs 6.84 ± 5.14 P=0.02). The serum iron concentration had a negative relationship with catalase (-0.599; P=0.04) and a positive relationship with glutathione peroxidase (0.729; P=0.007).

Conclusion: The iron serum levels increase could induce oxidative damage in pregnancy. Increased hepcidin is a useful biomarker for determining iron availability in pregnancy and its association with antioxidant systems.

Key words: *hepcidin, iron, oxidative stress, pregnancy,*

Introduction

Between 2% and 5% of pregnancies in women older than 30 are associated with metabolic disorders[1] and 40.1% are related to anemia-associated nutritional deficits, being 50% of these due to iron deficiency.[2] Therefore, the prophylactic prescription of iron supplements has become routine in the gestational period,[3] recommending from 30 to 60 mg of iron/day as a prophylactic dose during pregnancy to avoid anemia.[4]

The Mexican Institute of Social Security (IMSS) defined in 1959 a standardized supplemental dose of 60 mg of elemental iron, generally supplied as 200 mg of ferrous sulfate/day based on estimates of pregnancy iron requirements between 3.5 to 4 mg/day. Moreover, the Mexican government recommends the administration of iron and folic acid as prophylactic prescription.[2]

It is well-known that iron participates in the generation and propagation of reactive oxygen species (ROS) and lipid hydroperoxides, which play an

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important role in the pathophysiology of diseases such as gestational diabetes mellitus (GDM) and preeclampsia.[5]

It is recognized that, to guarantee an absorption of between 4 and 7 mg/day of iron, at least 20 mg/day of this element should be consumed in the diet (20% bioavailability), and adding 60 mg/day of supplemented iron, a pregnant woman would receive 80 mg/day of which 16 mg/day would be absorbed, which implies a dose 4 to 8 times higher than the minimum requirement amount, leading possibly to an iron overloading exposition in the gastrointestinal system. Hence, iron overload results in an increase in total body iron stores, a situation that could favor oxidative stress in any system and in the body in general.

Hepcidin peptide plays an important role in iron homeostasis since it regulates both iron absorption in the duodenum and its recycling process from senescent erythrocytes.[6] The aim of this work was to evaluate the correlations among serum iron levels, hepcidin and oxidative stress in pregnant women with or without metabolic disorders.

Methods

Study design

This cohort study, carried out at the "Mónica Pretelini Sáenz" Maternal Perinatal Hospital (HMPMPS), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, included pregnant women divided into two groups: A) women with low-risk pregnancy (WLRP), B) women with high-risk pregnancy (WHRP): women with metabolic disorders (dyslipidemias, GDM and high blood pressure).

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 22 subjects per group were necessary to recognize as statistically significant a difference greater than or equal to 3 units for hepcidin levels with a common standard deviation of 3.5.

The protocol was reviewed and approved by the HMPMPS Ethics in Research Committee (number: 2018-10-608) and the volunteers signed an informed consent letter. All the procedures were conducted in accordance with the Declaration of Helsinki (Fortaleza, Brazil) and the General Health Law of Mexico.

Inclusion and exclusion criteria

The inclusion criteria were pregnant women between 18 and 40 years old in the 2nd trimester of gestation prescribed with ferrous sulfate (60 mg/day), presence of metabolic alterations for the risk group pregnancy (previously diagnosed by the treating physician based on: hypertension: systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg): GDM; glucose > 130 mg/dL; dyslipidemia: cholesterol > 200 mg/dL and triglycerides > 150 mg/dL) or women with Low-Risk for control group. Pregnant women with congenital heart defects, uterine abnormalities, chronic degenerative diseases, pregnancies with congenitally abnormal fetuses, pregnancies with assisted reproductive technologies, history of smoking, type 2 diabetes mellitus (T2DM) and infectious, inflammatory or autoimmune diseases were excluded. Volunteers who abandoned the study or who were lost to follow-up were discarded from the final analysis.

Data Source

A questionnaire completed by all the patients gathered their health status and sociodemographic data including information of weight, height, blood pressure, blood chemistry (glucose, cholesterol and triglycerides) and blood biometry.

Blood samples were obtained in the 2nd (14-27 weeks of gestation) and 3rd trimester (28-42 weeks of gestation). The antioxidant activity was evaluated through the enzymatic quantification of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) using the methods of Radis et al.[7] Misra, et al.[8] Paglia et al.[9] respectively, as well as lipid peroxidation (LPOx) levels (Buege method).[10] The iron profile included serum iron, total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC) and iron saturation (%S). Ferritin was also measured by chemiluminescence and hepcidin quantification was done by sandwich ELISA (LifeSpan BioSciences, Inc.)

Sociodemographic data was analyzed by descriptive statistics. The medians of the biomarkers under study were compared using the Mann Whitney U test and in order to analyze the correlation among biomarkers, a Spearman correlation was performed. The IBM STPSS Statistics 22 program was used, setting a P value ≤ 0.05

as statistically significant.

Results

Of 106 patients who met the inclusion criteria in the 2nd. trimester of gestation, 42 were discarded, 20 did not want to sign the informed consent and 22 had inconsistent information. 64 volunteer patients, 23 in the WLRP group (mean age 23.8 ± 4.5 years, range: 18-31) and 41 in the WHRP group (27.0 ± 5.8 years, range: 18 to 31) participated in the study. Table 1 shows the population characteristics.

In relation to blood chemistry (Table 2), glucose levels were significantly higher by 7.93% in the WHRG group compared with the WLRP group only in the 2nd trimester. Cholesterol values in the WHRP group were significantly higher in the 2nd and 3rd trimester compared with those obtained in the WLRP group, by 19.7% and 17.3%, respectively.

The triglyceride values in the 2nd and 3rd trimester of the WHRP group were 1.67 and 1.65 times higher than those of the WLRP group. In the WLRP group, 26% exceeded the reference values for cholesterol in the 2nd trimester and 47.8% in the 3rd trimester. The triglyceride levels of 26% of the WLRP group were higher than 200 mg/dL in the 2nd trimester and 73.9% in the 3rd trimester. The WHRP group exceeded the 73.2% reference value for cholesterol in the 2nd trimester and 78.0% in the 3rd trimester. Likewise, for triglyceride, these percentages were 87.8% and 97.46%.

The results of the oxidative stress biomarkers show that SOD activity increased significantly between the 2nd and 3rd trimester in both groups (WLRP 26% and WHRP 21.9%); the increments for GPx in the same order were 23.7% and 23.2%. Finally, LPOx levels increased 34% in the WLRP group, but only 8.5% in the WHRP group without being significant.

Regarding iron profile, there was no significant difference between the study groups, nor between the trimesters of pregnancy. Notwithstanding, a higher hepcidin concentration was obtained in the WHRP group compared with the WLRP group for both the 2nd and 3rd trimester (23.2 and 46.6%, respectively). TIBC in the 3rd trimester of the WLRP group and in the 2nd and 3rd trimester of the WHRP group were higher than the reference values (240 to 450 µg/dL).

To analyze the results of the iron profile, the groups were formed as follows: WLRP (n = 11) and WHRP (n = 12) (since only these volunteers had all the determined parameters). A significant difference between the groups was observed only for iron saturation in the 3rd trimester (Table 3).

Finally, Spearman's correlation (Table 4) showed as main results that serum iron concentration had a significant negative relationship with CAT (-0.599; P=0.04) and positive with GPx (0.729; P=0.007). A positive correlation was obtained between SOD and LPOx (0.395; P=0.007), being lower for the WLRP group (0.481; P=0.24). It was also observed that at a lower hepcidin concentration, the SOD activity in the 3rd trimester of the WLRP group was higher (-0.6; P=0.050), and in the WHRP group the behavior was the opposite in the 2nd trimester of pregnancy. Interestingly, when the hepcidin concentration increased, the SOD activity also increased (0.615; P=0.033). For GPx activity, the correlation was negative with hepcidin (-0.636; P=0.026).

Table 1. Population characteristics

Variable	WLRP (N = 23) Mean ± SD		WHRP (N = 41) Mean ± SD	
	2nd trimester	3rd trimester	2nd trimester	3rd trimester
Age (years)	23.8 ± 4.5	-	27.0 ± 5.8	-
Height (m)	1.58 ± 0.07	-	1.57 ± 0.07	-
Weight (kg)	66.7 ± 11.1	70.3 ± 11.05	72.4 ± 14.8	75.2 ± 14.6
BMI (kg/m2)	26.4 ± 3.9	27.8 ± 3.7	29.2 ± 5.02	30.4 ± 4.94
Gestation (weeks)	22.8 ± 3.9	32.7 ± 2	23.2 ± 3.9	32.6 ± 2.1
Systolic Blood Pressure (mm Hg)	103.3 ± 8.4	106.9 ± 7.0	111.4 ± 13.5	111.9 ± 10.0
Diastolic Blood Pressure (mm Hg)	63.6 ± 8.2	63.9 ± 5.8	66.7 ± 9.6	69.5 ± 9.5

Abbreviations: BMI: Body Mass Index, WLRP: Women with Low-Risk, WHRP: Women with High-Risk Pregnancy.

Table 2. Means of the results of blood chemistry, oxidative stress and hepcidin (N = 64)

Variable	WLRP (N = 23)		WHRP (N = 41)		Mann Whitney U test			
	2nd trimester	3rd trimester	2nd trimester	3rd trimester	*	†	‡	§
Glucose (mg/dL)	83.7 ± 10.8	87.1 ± 11.4	91 ± 15.9	89.2 ± 13.5	0.206	0.026	0.568	0.711
Cholesterol (mg/dL)	187.5 ± 19.67	206.1 ± 37.9	233.5 ± 51.6	249.1 ± 62.4	0.15	<0.001	0.297	0.002
Triglycerides (mg/dL)	144.5 ± 33.71	193.5 ± 57.8	242.8 ± 72.6	318.8 ± 98.9	0.002	<0.001	<0.001	<0.001
SOD (µmol/g Hb)	470.2 ± 293.63	635.7 ± 253.4	432.2 ± 264.6	553.3 ± 274.3	0.049	0.68	0.043	0.26
CAT (nmol/g Hb)	570.1 ± 265.53	580.2 ± 314.2	475.8 ± 262.9	549.4 ± 295.1	0.852	0.177	0.301	0.828
GPx (µmol/g Hb)	14.0 ± 6.2	18.4 ± 7.9	13.3 ± 6.6	17.3 ± 6.2	0.049	0.45	0.007	0.506
LPOx (nmol/g Hb)	313.7 ± 185.25	475.5 ± 238.4	375.1 ± 156.7	410.1 ± 159.9	0.024	0.173	0.537	0.367
Hepcidin (ng/mL) n = 38	3.6 ± 4.2	3.6 ± 3.4	4.7 ± 3.2	6.8 ± 5.1	0.806	0.005	0.199	0.02

Abbreviations: CAT: catalase, GPx: glutathione peroxidase, LPOx: lipid peroxidation, SOD: superoxide dismutase, WLRP: Women with Low-Risk, WHRP: Women with High-Risk Pregnancy.

* WLRP 2nd trimester vs. WLRP 3rd trimester.

† WLRP 2nd trimester vs. WHRP 2nd trimester.

‡ WHRP 2nd trimester vs. WHRP 3rd trimester.

§ WLRP 3rd trimester vs. WHRP 3rd trimester.

Table 3. Means of the results of iron profile and hepcidin (n = 23).

Variable	WLRP (N = 11)		WHRP (N = 12)		Mann Whitney U test			
	2nd trimester	3rdtrimester	2nd trimester	3rd trimester	*	†	‡	§
Iron (µg/dL)	78.53 ± 33.73	86.54 ± 32.38	64.43 ± 18.75	69.6 ± 19.76	0.438	0.487	0.514	0.316
TIBC (µg/dL)	439.35 ± 80.58	437.15 ± 81.58	463.47 ± 98	460.23 ± 97.85	0.949	0.487	0.799	0.525
UIBC (µg/dL)	356.45 ± 94.19	358.54 ± 90.45	397.4 ± 90.73	392.9 ± 92.83	0.898	0.19	0.843	0.288
Iron saturation (%)	16.9 ± 7.12	20.35 ± 6.81	14 ± 4.26	15.08 ± 5.33	0.217	0.169	0.478	0.016
Ferritin (ng/mL)	15.98 ± 12.45	18.92 ± 14.97	9.62 ± 2.94	11.55 ± 3.4	0.519	0.413	0.198	0.288
Hepcidin (ng/mL)	2.61 ± 3.12	3.38 ± 3.72	4.64 ± 3.69	7.17 ± 6.43	0.519	0.104	0.478	0.118

Abbreviations: TIBC: total iron-binding capacity, UIBC: unsaturated iron-binding capacity, WLRP: Women with Low-Risk pregnancy, WHRP: Women with High-Risk Pregnancy.

* WLRP 2nd trimester vs. WLRP 3rd trimester.

† WLRP 2nd trimester vs. WHRP 2nd trimester.

‡ WHRP 2nd trimester vs. WHRP 3rd trimester.

§ WLRP 3rd trimester vs. WHRP 3rd trimester.

Table 4. Correlation of biomarkers of iron profile and hepcidin with blood chemistry and oxidative stress

Variables	WLRP and WHRP (N = 23)		WLRP 2nd trimester (N=11)		WLRP 3rd trimester (N=11)		WHRP 2nd trimester (N=12)		WHRP 3rd trimester (N=12)	
	r	P value	r	P value	r	P value	r	P value	r	P value
Iron - TIBC									0.608	0.036
Iron - UIBC	-0.325	0.028								
Iron - %S	0.815	<0.001	0.918	<0.001	0.9	<0.001	0.76	0.004	0.579	0.049
Iron - Ferritin			0.818	0.002	0.818	0.002				

Cont... **Table 4. Correlation of biomarkers of iron profile and hepcidin with blood chemistry and oxidative stress**

Iron - CAT							-0.599	0.04		
Iron - GPx							0.729	0.007		
%S - TIBC	-0.407	0.005								
%S - UIBC	-0.646	<0.001	-0.683	0.02	-0.691	0.019				
%S - Ferritin			0.782	0.004	0.745	0.008				
%S - Glucose					-0.627	0.039				
%S - Triglycerides							-0.643	0.024		
Ferritin - UIBC			-0.756	0.007	-0.645	0.032				
Ferritin - Cholesterol					-0.645	0.032				
Ferritin - Triglycerides			0.645	0.032						
Ferritin - CAT	-0.332	0.024								
Ferritin - LPOx									-0.632	0.028
TIBC - UIBC	0.92	<0.001	0.902	<0.001	0.845	0.001	0.839	0.001	0.734	0.007
TIBC - Cholesterol					0.8	0.003				
TIBC - Triglycerides									0.651	0.022
UIBC - Cholesterol					0.673	0.023				
UIBC - Triglycerides							0.58	0.048		
Hepcidin - SOD					-0.6	0.05	0.615	0.033		
Hepcidin - CAT	-0.316	0.032								
Hepcidin - GPx									-0.636	0.026

Abbreviations: CAT: catalase, GPx: glutathione peroxidase, LPOx: lipid peroxidation, SOD: superoxide dismutase, TIBC: total iron-binding capacity, UIBC: unsaturated iron-binding capacity, %S: iron percentage saturation, WLRP: Women with Low-Risk pregnancy, WHRP: Women with High-Risk Pregnancy.

Discussion

The results of this study showed a similar increase in cholesterol and triglycerides in both groups of volunteers from the 2nd to the 3rd trimester of pregnancy. Similar results to previous published information.[11,12,13] In this survey, 34.1% of pregnant women in the WHRP group were hypertensive and 51.2% were dyslipidemic.

On the other hand, SOD and GPx levels increased significantly from the 2nd to the 3rd trimester of pregnancy, in both groups, probably to offset the effect of lipid peroxides and other free radicals produced by abnormal lipid metabolism and inflammation processes

present when metabolic disturbances occur during pregnancy.[12]

Regarding the iron profile, it was found that the volunteers of both groups presented normal serum iron levels (60 to 170 µg/dL). However, the TIBC in the 3rd trimester of the WLRP group and in the 2nd and 3rd trimester of the WHRP group were higher than the reference values (240 to 450 µg/dL),[14] which would indicate the absence of iron deficiency in both groups, and that by increasing the serum iron concentration, it also increases the binding capacity of transferrin and binding sites. This hypothesis is reaffirmed by the positive relationship between serum iron and iron saturation values,[15] as well as a negative relationship between UIBS and iron saturation.

Overall, the positive relationship between iron levels and TIBC in the 3rd trimester in the WHRP group would indicate a probable iron overload when the binding capacity with transferrin saturates, leading to non-transferrin-bound iron being internalized in tissues,[16] where the ROS would increase, unbalancing the antioxidant systems, reflected in the negative relationship between serum iron with CAT and the positive relationship with GPx, as well as the formerly described increase in GPx, affecting insulin secretion and lipid oxidation, resulting in increased sensitivity to insulin and predisposition to GDM.[17]

It is worth noting that excess free iron can accept and donate electrons not only to catalyze the Fenton and Haber-Weiss reaction, but also to propagate free radical chain reactions, acting as an oxidative substance with a role in endothelial destruction, and thus participating in the pathogenesis of preeclampsia or GDM,[18] pathologies present in the WHRP group. Usually, efficient iron mobilization of reserves is reflected by lower concentrations of ferritin in the 3rd trimester of pregnancy,[19] contrary to our results in both groups, in which we found an increase, although not significant.

When analyzing hepcidin levels, a significant increase from the 2nd to the 3rd trimester, in both groups, was found, data consistent with those reported early in high-risk pregnancies associated with inflammatory conditions (GDM or preeclampsia), in which hepcidin rises compared with healthy pregnancies, suggesting a probable iron accumulation as a result of the sulfate ferrous prescription, since when finding a state of poor iron in pregnancy low levels of hepcidin have been found,[20] being the lowest during the 3rd trimester compared with the 1st and 2nd trimester, allowing maximum iron transfer to the fetus.[21]

The previous result points to the relevance of measuring hepcidin during pregnancy. For example, finding low levels of it could identify pregnant women who need iron supplementation before other iron status parameters, like hemoglobin change.[19] Conversely, in this cohort, the two patients with the highest values of hepcidin developed preeclampsia.[19]

In support of the notion of the importance of hepcidin is the finding of a negative relationship between this hormone and SOD in the WLRP group, reflecting the increase in hepcidin expression due to the increment in systemic iron, which in turn helps to reduce iron-mediated oxidative stress by increasing SOD activity,[14] causing an increase of LPOx levels, without implying metabolic damage when negatively related to glucose.

In the WHRP group, antioxidant activity was affected by iron, a situation reflected by the positive relationship of hepcidin with SOD, causing greater metabolic damage in the 3rd trimester of pregnancy when presenting an increase in the levels of GPx described before, a positive relationship between cholesterol with GPx and LPOx, as well as glucose levels with triglycerides, coinciding in part with a previously reported relationship between triglycerides and SOD in patients with preeclampsia where it would increase the risk of vascular disorders that trigger endothelial dysfunction, atherosclerosis and thrombosis,[12] so that oxidative stress and insufficiency in antioxidant defense systems could be factors that lead to an increase in lipid peroxidation in metabolic diseases such as preeclampsia and GDM,[22] in addition to previously described elevated serum concentrations of ferritin and hepcidin, which lead to insulin resistance due to sensitization of peripheral glucose receptors.[23]

A previous study in Mexico evaluated the intake of 60 mg of iron, finding that it caused elevations in hemoglobin, serum ferritin, iron, and LPOx, demonstrating that excessive iron intake during pregnancy could be one of the causes of cellular damage.[24] Iron overload has also been evaluated in mouse studies, demonstrating that it can generate a pathology similar to that observed in

T2DM, leading to hyperglycemia, hyperinsulinemia, lipid synthesis induction and insulin resistance, as well as increased production of proinflammatory cytokines, such as interleukin (IL)-6, which induces the hepcidin gene transcription.[25]

It can be concluded from this study that in both circumstances, High-Risk and Low-Risk pregnancies, an increase in systemic iron levels can occur due to an overload induced by iron supplements without a confirmed diagnosis of anemia. The possibility exists that in women with Low-Risk, despite the fact that the antioxidant enzyme systems are responding to the presence of ROS, they are not totally effective since there is a significant increase in LPOx of the 2nd to the 3rd trimester of pregnancy.

A limitation of this study is the small number of patients. Notwithstanding, a final important message is that hepcidin measurement in pregnancy is important as it is the regulating hormone of iron homeostasis, and may be a useful biomarker to determine the availability of iron in pregnancy and its association with antioxidant systems could establish whether the increase in serum iron is one of the factors that lead to the development of metabolic disorders such as preeclampsia, GDM and dyslipidemia.

Acknowledgement: This project was partially funded by the Autonomous University of the State of Mexico (UAEMéx) and by Ciprés Grupo Médico.

Conflicts of Interest: All authors have disclosed no conflicts of interest.

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