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Hepcidin levels in Mexican pregnant women without family preeclampsia

Research Article

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Abstract: Hepcidin is a primary regulator of iron metabolism in the human body by promoting ferroportin degradation, hepcidin reduces intestinal iron absorption and its release from intracellular stores but regarding its role in the development of preeclampsia, information is scarce and contrasting. It is hypothesized that increased hepcidin may contribute to the inflammatory cascade leading to preeclampsia. The aim of this study was to determine whether serum hepcidin levels increase in pregnant women without family history of preeclampsia. This was a cohort study including pregnant women of any age, from the second trimester. Blood samples were drawn and routine analyses were performed for blood cytometry. Hepcidin quantification was done using sandwich ELISA. Based on the Gaussian distribution of the variables, either Pearson or Spearman correlation were used among the variables. Thirty-three pregnant women were recruited, mean age 23.8 ± 9.1 years, the mean hepcidin levels were 5.17 ± 0.04 μg/l. The following risk factors to have a complicated pregnancy were recorded: overweight or obesity: 11 (33.33%), first pregnancy: 6 (18.18), alcoholism: 2 (6.06%), multiple pregnancy: 2 (6.06%), drug addiction (crystal): 1 (3.03%), epilepsy: 1 (3.03%), taking steroids: 1 (3.03%) and epilepsy: 1 (3.03%). There were four patients without any risk factor. The Spearman correlation test was used (after performing the Kolmogorov test, hepcidin showed a non-parametric distribution) but no positive or negative correlation was obtained between the included variables and hepcidin. It can be concluded that in pregnant patients without family history of preeclampsia and who do not develop this obstetric complication, hepcidin levels remain within the normal range for the reference population. The implication is that hepcidin measurements in the reference range may help monitor a normally developing pregnancy.

1. Introduction

In recent years, special attention has been paid to the metabolism of iron. Socio-economic changes in diet have impacted in one way or another on its homeostasis in our body, but it is also necessary to mention that the increase in pathologies is related both to its deficit, such as iron deficiency anemia, as well as its excess (Mégier et al., 2022; Li et al., 2023).

Iron deficiency diagnosis is a key health priority during pregnancy. The precise determination of indicators is needed for the evaluation of iron deficiency. According to the World Health Organization

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(WHO), iron deficiency anemia globally accounts for 45% in the most vulnerable groups of pregnant women and infants (<5 years old). Recently, the efficacy of iron replacement therapy and the effectiveness of current standard testing of iron parameters have been reviewed in order to evaluate whether a more accurate diagnosis can be made using alternative and/or supplementary markers (Ortega López et al., 2020).

Hepcidin is a primary regulator of iron metabolism in the human body. By promoting ferroportin degradation, hepcidin reduces intestinal iron absorption and its release from intracellular stores (Figure 1).

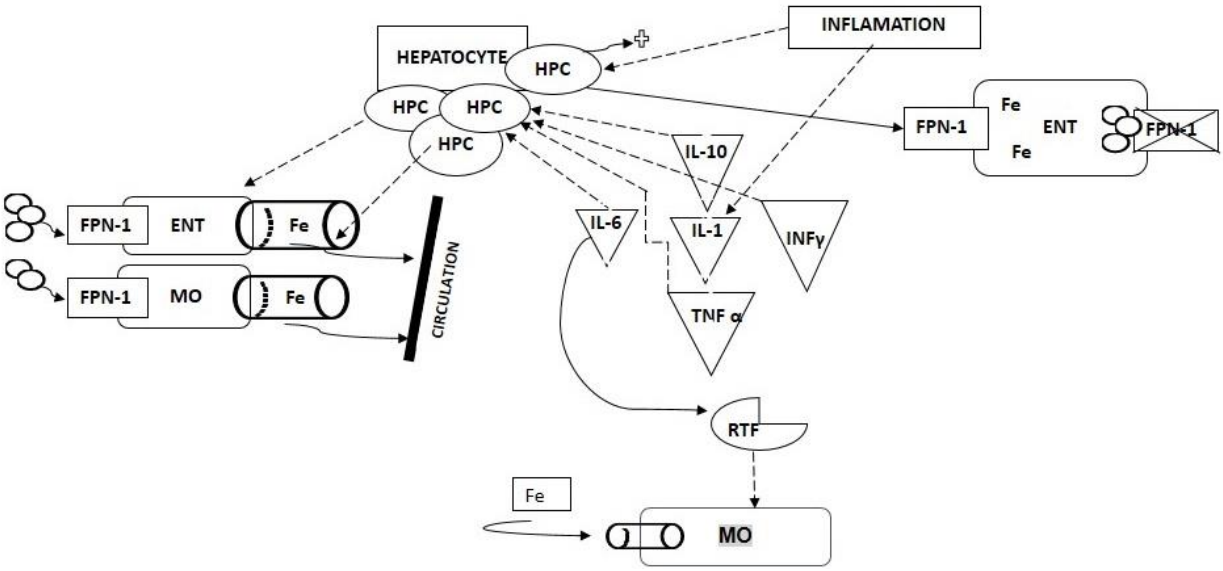

Figure 1. ENT: enterocyte, Fe: iron, FPN-1: ferroportin, HPC: hepatocyte, IL: interleukin, INF-γ: Interferon-gamma, MO: macrophages, RTF: transferrin receptor, TNF-α: Tumor necrosis factor alpha. Hepcidin is produced by hepatocytes. Some cells such as enterocytes and macrophages have a receptor called ferroportin in their membrane, which allows the passage of the hepcidin protein so that iron reserves are released into the circulation and thus, it can be used for several functions. In the face of inflammatory processes, a cascade of interleukins is activated involving IL-1, IL-6, IL-10, INF-γ and TNF-α, which causes an increase in the interaction with transferrin receptors, inducing macrophages to capture a greater amount of iron. During this process, due to the aforementioned cascade, hepcidin production is accelerated; then it migrates into the cell leading to ferroportin degradation through internalization, thus causing the iron to not be able to leave the cell and its reserves to increase exponentially.

There is clear evidence that supports the valid role of hepcidin in the diagnosis of iron deficiency anemia in pregnant women (Aringazina et al., 2023). For example, in the course of pregnancy, gradually declining hepcidin concentrations encourage placental iron transfer, thereby providing the appropriate amount of iron for fetal development (Wojciechowska et al., 2022).

The most recent studies have shed more light on serum hepcidin and raised questions about the significance of pregnancy-related inflammatory markers, including cytokines in iron deficiency anemia (Chibanda et al., 2023). Regarding its role in the development of preeclampsia, information is scarce and contrasting (Cardaropoli et al., 2018; Ahmed et al., 2023). It is hypothesized that increased hepcidin may contribute to the inflammatory cascade leading to preeclampsia. If this is so, it means that it would be

necessary to rationalize that excessive iron intake during pregnancy could be harmful by being a promoter of the inflammatory cascade, as well as its deficiency (anemia). The aim of this study was to determine whether serum hepcidin levels increase in pregnant women without a family history of preeclampsia.

2. Materials and methods

2.1. Patients

This cohort study, carried out in the months of March and April 2021 at the "Mónica Pretelini Sáenz" Maternal Perinatal Hospital (HMPMPS), Health Institute of the State of Mexico (ISEM), Toluca, Mexico, included pregnant women. The inclusion criteria were pregnant women of any age, from the second trimester.

The sample was calculated with the next formula:

$$n = \frac{Z\alpha S^2}{d^2}$$

Where n_0 is the sample size for an infinite population, Zα is a value associated with the level of confidence (1.96), S is the standard deviation (1.1) and d is the maximum permissible error (0.07). In this survey the result was: 33.88

Pregnant women with assisted reproductive technologies, type 2 diabetes mellitus or with active infection were excluded. Volunteers who abandoned the study or who were lost to follow-up were discarded from the final analysis.

2.2. Data Source

The institutional questionnaire was completed by all the patients, gathering their health status and sociodemographic data, including information of weight, height, blood pressure, and blood biometry.

2.2.1. Laboratorial studies

Blood samples were obtained after 8 hours of fasting. Blood samples were drawn, and routine analyses were performed for blood cytometry (Advia 120, Bayer Health). Hepcidin quantification was done by sandwich ELISA (LifeSpan BioSciences, Inc.) on an ELx800 ™ device (BioTek Instruments, Inc.) at the Research Laboratory of Ciprés Grupo Médico S.C. (CGM). A calibration curve was made for each measurement and the equipment received maintenance service before the project.

2.2.2. Ethics

The protocol was reviewed and approved by the HMPMPS Ethics in Research Committee (number: 2021-06-738) 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.

2.2.3. Statistical analysis

The descriptive statistics were obtained using Excel. Quantitative variables were represented by measures of central tendency. First, the Kolmogorov test was performed to determine the normality of the variables. Based on the Gaussian distribution of the variables, either Pearson or Spearman correlation was used among the variables. In all cases, $p \leq .05$ was considered statistically significant. The statistical

analyses were carried out using the free Social Science Statistics web (socscistatistics.com, last updated: November 2018).

3. Results

During the period of follow up 33 pregnant women were recruited, with a mean age 23.8 ± 9.1 years. The age distribution was as follows: less than 18 years old: 14 (42.42%), from 18 to 30: 10 (30.3%), over 30 years old: 9 (27.27%) (The main results are shown in Table 1).

Table 1. Characteristics of the patients

Variable	(Mean ± SD)
Age (years)	23.8 ± 9.1
BMI (kg/m²)	25.9 ± 4.8
Gestational age (weeks)	18.8 ± 4.7
MAP (mmHg)	108.6 ± 13.45
Hb (g/dL)	13.73 ± 1.16
Hematocrit (%)	40.48 ± 2.68
Erythrocytes (million cells/µL)	4.56 ± 0.32
MCV (fl)	86.98 ± 12.07
Hepcidin (µg/l)	5.17 ± 0.04

BMI: Body Mass Index, Hb: hemoglobin, MAP: Mean Arterial Pressure, MCV: Mean corpuscular volume, SD: Standard Deviation.

In this group of patients, the following risk factors to have a complicated pregnancy were recorded: overweight or obesity: 11 (33.33%), first pregnancy: 6 (18.18), alcoholism: 2 (6.06%), multiple pregnancy: 2 (6.06%), drug addiction (crystal): 1 (3.03%), epilepsy: 1 (3.03%), taking steroids: 1 (3.03%) and epilepsy: 1 (3.03%). There were four patients without any risk factors.

After performing the Kolmogorov test, hepcidin showed a non-parametric distribution, so the Spearman correlation test was used, but no positive or negative correlation was obtained between the included variables and hepcidin (Table 2).

Table 2. Spearman correlation

Variable correlated with hepcidine	rs	p (2-tailed)
Age (years)	-0.13549	0.45218
BMI (kg/m²)	-0.03477	0.84768
Gestational age (weeks)	-0.10846	0.54796
MAP (mmHg)	-0.03226	0.85856
Hb (g/dL)	0.31853	0.07082
Hematocrit (%)	0.18595	0.30015
MCV (fl)	0.12201	0.49878

BMI: Body Mass Index, Hb: hemoglobin, MAP: Mean Arterial Pressure, MCV: Mean corpuscular volume, SD: Standard Deviation.

4. Discussion

A previous systematic review and meta-analysis evaluated the correlation between maternal hepcidin and other biomarkers of iron status, markers of inflammation, and maternal body weight during

pregnancy, as well as neurodevelopment in the offspring. The conclusion was that hepcidin weakly to moderately correlated with biomarkers of iron and inflammation in pregnancy (Ssewanyana et al; 2023).

Another systematic review and meta-analysis that included 760 individuals showed that mean hepcidin levels were significantly higher in women who developed preeclampsia as compared to women who did not develop this complication (Bandyopadhyay et al., 2022). Our study agrees with this report in that none of our patients developed preeclampsia and hepcidin levels remained normal.

Interestingly, data suggest that hepcidin values are higher in pregnant women at high altitude than pregnant women at low altitude, and probably this response helps to adequate iron status during pregnancy as reflected by higher serum ferritin levels and lower soluble transferrin receptor levels (Figueroa-Mujica et al., 2022). This is a window of opportunity for the future since the city of Toluca, where this study was conducted, is located 2,600 meters above sea level.

Variations in hepcidin levels between non-iron deficient, iron deficient, and with iron deficiency anemia women are uncovered, and it is reported that the lower hepcidin levels diagnosed in iron deficiency anemia are closely linked to hemoglobin in Pakistani women. The group that made this statement concluded that hepcidin can be a valuable marker in identifying iron deficiency and iron deficiency anemia during the second trimester of pregnancy (Arshad et al., 2021).

A very important fact is that iron supplementation in preeclampsia patients might have led to a state of iron overload, which might have caused oxidative stress and endothelial dysfunction in preeclampsia patients (Figure 2). The rise in hepcidin levels in this scenario may be viewed as a protective mechanism to combat iron overload mediated cytotoxicity (Shaji Geetha et al., 2022).

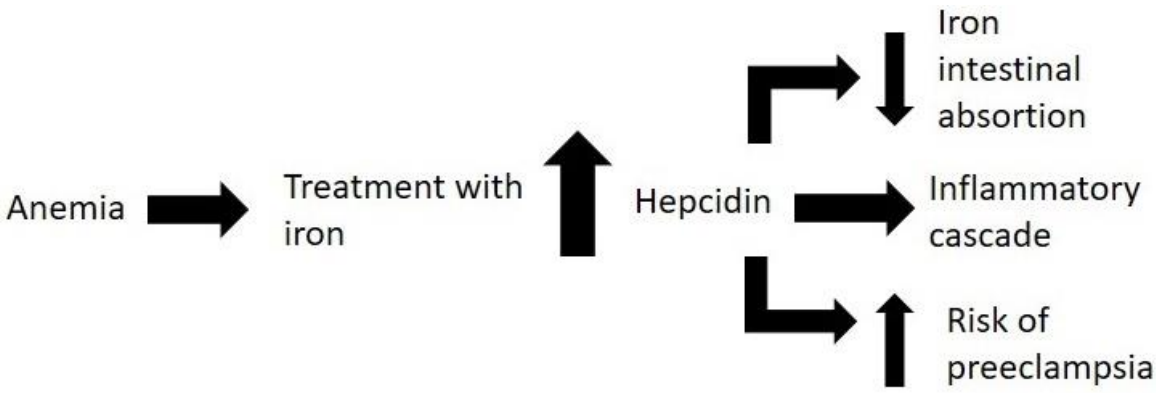

Figure 2. Anemia treatment in pregnancy, hepcidin and risk of preeclampsia.

Most recently, it has been postulated that erythroferrone (ERFE), a new marker and hormonal regulator of iron, is expressed in trophoblasts, with lower levels in early-onset preeclamptic placentas than in those from healthy pregnancies. In addition, maternal plasma ERFE levels were elevated in both early- and late-onset preeclampsia, and hepcidin levels were reduced in early-onset preeclampsia. At the same time, maternal plasma interleukin-6 (IL-6) levels were unaltered, suggesting that in this situation, inflammation was not involved in altered iron regulation in preeclampsia cases (Masoumi et al., 2023). Contrary to what was mentioned previously, another study showed that maternal hepcidin levels were found to be significantly elevated in preeclampsia patients as well as in mothers with poor fetal outcomes (Nila et al., 2021).

The role of hepcidin in iron homeostasis in preeclamptic pregnant women is unclear. Analyzing hepcidin concentration, dietary iron intake, hematological indices, iron status, liver function, and inflammatory markers in 18 preeclamptic women and 18 healthy normotensive pregnant women of

similar age, in an observational case-control study, hematocrit was significantly higher in the preeclamptic group compared with the control, whereas erythropoietin level was significantly lower. The pronounced inflammatory status of preeclamptic women was confirmed by significantly higher concentrations of IL-6, tumor necrosis factor-α (TNF-α), and ferritin. Nonetheless, the preeclamptic group exhibited significantly higher serum iron and transferrin saturation, and these alterations were accompanied by lower hepcidin levels. No significant correlations between hepcidin concentration and iron status parameters were observed in either group. However, a positive and significant correlation between hepcidin concentration and C-reactive protein was observed in the preeclamptic group, concluding that high serum iron in preeclamptic women is likely caused by low production of hepcidin (Brunacci et al., 2018). The results of this study are contrary to the majority of articles that talk about preeclampsia being correlated with higher levels of hepcidin.

A limitation of this study is the small number of patients included, which restricts its generalization, but we believe that having achieved the measurement of a variable that is not routine means a contribution worth taking into account.

The direct implication of our findings is that hepcidin measurements in the reference range may help monitor a normally developing pregnancy. Otherwise, an increase in its levels would indicate a risk since it is known to be a co-conductor of the inflammatory cascade.

5. Conclusion

From the information obtained from this study and that found in the literature, it can be highlighted that there is a possibility that, in the case of excessive treatment with iron during pregnancy, some patients develop an inflammatory cascade and are at greater risk of developing preeclampsia. Also, it can be concluded that in pregnant patients without a family history of preeclampsia and who do not develop this obstetric complication, hepcidin levels remain within the normal range for the reference population.

It could be proposed that the measurement of hepcidin together with iron kinetics would provide very useful information during pregnancy, not only to evaluate its decrease but also to detect cases in which it increases significantly, as it could be a warning of iron overdose and even risk of preeclampsia.

Author contributions

Conceptualization, Mendieta Zerón H.; methodology, software, validation, Cano Murillo L.M., Ramírez Figueroa M., Sánchez Fragoso L.X. & Layton Tovar C.; formal analysis, investigation, Cano Murillo L.M., & Layton Tovar C.; data curation, writing—original draft preparation, all authors; writing—review and editing, Mendieta Zerón H.; visualization, supervision, project administration, funding acquisition, Mendieta Zerón H.

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Data availability

Data are available upon the request.

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Conflicts of interest

The authors declare no competing interests.

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