Comparative analysis of iron status and other hematological parameters in preeclampsia†

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ABSTRACT

Objective: To compare serum ferritin (SF) concentrations and other hematological parameters between patients with preeclampsia (PE) and normal pregnant women of the same gestational period who received supplemental iron during pregnancy.

Methods: Prospective, comparative, observational pilot study that included 31 women with PE and 30 healthy pregnant women, at 20 weeks’ gestation. Ferritin, iron and complete blood cell count were compared between groups.

Results: In comparison with controls, preeclamptic patients had a higher weight, body mass index, and arterial pressure. Serum ferritin and serum iron were higher in patients with PE (median: 36.5 μg/l vs. 20.9 μg/l and 103.9 μg/dl vs. 90.8 μg/dl) with a significant difference (P = 0.019 and P = 0.345). SF values >40 μg/l correlated with PE (r = 0.281; P = 0.032). A platelet count less than 100 × 10^9/l was higher in the PE group than in the control group (13% vs. 3%, P = 0.354).

Conclusion: Higher SF levels, despite being within normal range, were associated with PE. The incidence of thrombocytopenia was higher in preeclamptic women, however, the remaining hematological parameters were similar in both groups.

Introduction

Preeclampsia (PE) is characterized by maternal hypertension and proteinuria and its pathophysiology involves virtually every system in the body. [1–3] This multifactorial disease affects 10% of pregnancies, representing a major cause of maternal and fetal mortality. [4] In Mexico, 34% of maternal mortality is caused by PE. [1,5] The etiology of PE remains unknown, however, many risk factors have been associated. [1,6,7] Despite the fact that there is no other cure for PE except delivery, early identification of high-risk pregnant women would lead to timely interventions to minimize the risk of complications. [1,4] Different laboratory abnormalities may be observed in patients with PE. Some have been studied as predictors for development of the disease, including platelet count, platelet indices (mean platelet volume (MPV) and platelet distribution width (PDW)), serum iron and serum copper, among others. On the other hand, there are many theories to explain PE pathogenesis such as abnormal placental implantation and production of angiogenic substances, [8] genetic factors, [9] interacting immunological factors [10] and increased oxidative stress. During pregnancy placental conditions favor the production of reactive oxygen species (ROS). [11] Lipid hydroperoxides are formed from peroxidative reactions, and these can be used as serum markers that reflect the state of oxidative stress. [12] When a pregnancy is complicated by PE, these serum markers are more elevated than in healthy pregnancies. [13] Elevated ROS have been associated with PE and gestational diabetes mellitus. [14] ROS are not sufficient to initiate cell damage directly; however, in the presence of catalytic amounts of iron, these can generate hydroxyl radicals by Fenton reaction and initiate the process of lipid peroxidation which may result in endothelial cell damage.

Some reports suggest that prophylactic iron supplementation may be harmful to pregnant women who are not iron deficient. [15] It is unlikely that this can cause iron overload; however, the increase in available iron and its storage could contribute or exacerbate oxidative stress. During pregnancy iron supplements...
are used independently of hemoglobin levels, especially in developing countries.\textsuperscript{16,17} In Mexico, iron is prescribed regardless of serum ferritin (SF) levels because ferritin is not usually measured in pregnant women. The aim of this study was to compare SF concentration and other hematological parameters between patients with PE and normal pregnant women without PE at the same gestational period who received supplemental iron during pregnancy.

Material and methods
In this prospective, comparative, observational pilot study, we invited pregnant patients who were admitted to the Obstetrics Service of the Hospital Universitario ‘Dr. José Eleuterio González’ with a diagnosis of PE and more than 20 weeks of gestation (WG), between October 2012 and May 2014 to participate. The control group consisted of healthy women over 20 WG coming to the hospital with labor contractions who were chosen sequentially according to date. The sample size was according to the number of patients who were chosen sequentially according to date. The sample size was according to the number of patients with PE admitted to the hospital in the indicated period with a PE: control ratio of 1:1. All included women had received oral iron prescribed by their treating physician as prophylaxis for iron deficiency anemia during pregnancy for more than 30 days. Women who did not sign the informed consent, had a gestational age less than 20 WG, were transfused with packed red blood cells in the last 30 days, had a previous diagnosis of hemochromatosis, chronic hypertension or an active infectious process were excluded. All patients that were invited agreed to be included. The protocol was approved by the local ethics committee of the Institutional Review Board and all patients signed informed consent forms before any procedure.

Definitions

PE was defined as pregnancy-induced hypertension (systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg on two occasions at least 6 hours apart) associated with proteinuria ($\geq 300$ mg/24 hours or $\geq 30$ mg/dL on two urine samples), with or without end-organ damage, arising after 20 WG. A control was defined as a healthy pregnant woman who went to the hospital with labor contractions and who did not have a diagnosis of PE. The normal reference range for SF was considered to be $15.0–150 \mu g/l$.

Gestational age was determined according to the last menstrual period and was confirmed with ultrasound at the time of hospital admission in all cases.

Procedures

All women were interviewed at enrollment. A detailed medical history was taken including information regarding prophylactic use of iron (start and end date, route of administration, dosage and indication), history of PE in previous pregnancies and blood pressure at the time of admission. Ten milliliters of peripheral venous blood were collected in two tubes. Five milliliters were placed in a citrated tube to perform a complete blood cell count using an Sysmex XT-4000i hematology analyzer (Sysmex Corporation, Hyogo, Japan), and 5 ml were placed in a tube without anticoagulant to determine the concentrations of ferritin and serum iron in a COBAS INTEGRA$^\text{®}$ processor 400 PLUS (Roche Diagnostics GmbH, Mannheim, Germany). In the control group all blood samples were taken before delivery. In the PE group, samples were taken after the diagnosis of PE, immediately before delivery.

Statistical analysis

IBM SPSS Statistics for Windows, version 20.0 software was used for statistical analysis. Demographic characteristics and laboratory results were described as means with their standard deviation or medians with ranges and/or percentages, with regard to normally or non normally distributed data, respectively. The Kolmogorov–Smirnov test was performed to determine normality. Hypothesis testing was performed to compare data collected from both groups using Student's $t$ test for parametric quantitative data in normally distributed data; otherwise, the Mann–Whitney $U$ test was used. Fisher's exact test was used on categorical data. Spearman's correlation coefficient was used to correlate SF with other maternal parameters. A $P$-value of $<0.05$ was considered statistically significant.

Results

Demographic characteristics

Sixty-one women were included in this study, 31 with PE and 30 healthy pregnant controls. No significant difference was found in age, height, days receiving iron or iron dose received in either group (Table 1). However, as is expected in patients with PE, this group had a higher weight, and a greater body mass index and mean arterial pressure than women in the control group ($P = 0.029$, $P = 0.013$ and $P < 0.001$, respectively). Moreover, unlike the control group, most of the patients in the PE group (58.1%) were in their first pregnancy and had fewer WG (36 $\pm$ 3.8 vs. 40 $\pm$ 1.1).

Ferritin and iron status

A ferritin level above the upper normal limit was observed in only four patients (13%) of the PE group and in one patient (3%) of the control group. However, there was a statistically significant difference
Table 1 General characteristics of the PE and normal pregnant groups

|                          | Preeclampsia group (n = 31) | Control group (n = 30) | P  |
|--------------------------|------------------------------|------------------------|----|
| Age (years)              | 25.7 ±7.5                    | 26.7 ±6.2              | 0.576 |
| Weight (Kg)              | 89 ±19.2                     | 79.3 ±14.2             | 0.029 |
| Height (m)               | 1.59 ±0.06                   | 1.60 ±0.07             | 0.931 |
| BMI (Kg/m²)              | 34.7 ±5.8                    | 31.2 ±4.8              | 0.013 |
| MAP (mmHg)               | 110.6 ±7.6                   | 83.8 ±7.1              | <0.001 |
| WG                       | 36 ±3.8                      | 40 ±7.1                | <0.001 |
| Days Fe+**               | 183.9 ±64.9                  | 205.8 ±79.4            | 0.269 |
| Total pregnancies        | 1 – 6                         | 3 1 – 6                | 0.02  |
| Daily doses Fe+ (mg)     | 60 – 115                     | 60 20 – 120            | 0.068 |
| Primigravidae            | 18 ±58.1                     | 6 20 0.004             |     |

*Days of Fe+ were calculated from the start date to the end date of iron intake.
BMI, body mass index; MAP, median arterial pressure, WG, weeks of gestation.

(P = 0.019) in the median values of SF between groups (Table 2) with higher levels in the PE group (36.5 vs. 20.9 μg/l, respectively). A sub-analysis of SF was performed considering a cutoff of 40 μg/l, noting that 45.2% of patients with PE and 20% in the control group were above this value, without reaching statistical significance (P = 0.054). Also it was found that a SF value >40 μg/l correlated with PE (r = 0.281; P = 0.032). Although the correlation is weak, statistical significance was observed. Similarly, serum iron was higher in patients with PE (103.9 vs. 90.8 μg/dl) (P = 0.345). A positive correlation between elevated iron levels and higher SF levels was observed (r = 0.297; P = 0.024) in both groups.

Hematological parameters

The incidence of thrombocytopenia, with a platelet count less than 100 × 10³/l, was higher in the group with PE than in the control group (13 vs. 3%, P = 0.354). However, no significant difference was found in the mean number of leukocytes, neutrophils, lymphocytes, or monocytes, hemoglobin levels, hematocrit, platelet count, platelet volume distribution, and platelet volume between both groups (Table 3).

Characteristics of newborns

When comparing the characteristics of neonates in both groups (Table 4), those of patients with PE had a lower weight (mean 2.555 vs. 3.455 g), were shorter (median 48 vs. 51 cm) and obtained a lower CAPURRO score (median 38.1 WG vs. 39.7 WG) than newborns of control women, with statistically significant differences in all of these parameters (P = <0.001). Regarding the APGAR score, 9.7% of newborns born from mothers with PE had a low score (<7 points) at 1 minute of life in the group born to mothers with PE, in contrast to 3.3% in the healthy mothers group (P = 0.612). With relation to APGAR scores at 5 minutes of life, 6.5% of newborns born from mothers with PE kept a low score compared to 3.3% of the control group (P = 1.0). Two newborns died in the PE group (6.5%) and none in the control group (P = 0.492). The deaths were due to prematurity and in both cases the mothers had more than 40 μg/l of SF.

Discussion

According to the World Health Organization (WHO), every 7 minutes a woman dies from PE. In developing

Table 2 Comparison of iron and ferritin levels between the PE and normal pregnant groups

|                          | Preeclampsia group (n = 31) | Control group (n = 30) | P  |
|--------------------------|------------------------------|------------------------|----|
| Iron (μg/dl)             | 103.9 ±65.16                 | 90.8 ±39.6             | 0.345 |
| Weight (Kg)              | 36.5 ±4.3 – 105.7            | 20.95 ±4.7 – 112.7     | 0.019 |
| Ferritin (μg/l)          | n= %                        | n= %                   |     |
| Ferritin >40 μg/l        | 14 45.2                      | 6 20                   | 0.054 |

Table 3 Comparison of the complete blood cell count between the PE and normal pregnant groups

|                          | Preeclampsia group (n = 31) | Control group (n = 30) | P  |
|--------------------------|------------------------------|------------------------|----|
| WBC (10³/μl)             | 9.77 ±3.23                   | 9.68 ±3.02             | 0.911 |
| NEU (10³/μl)             | 7.42 ±2.93                   | 7.48 ±2.79             | 0.944 |
| LYM (10³/μl)             | 1.62 ±0.58                   | 1.55 ±0.49             | 0.603 |
| MONO (10³/μl)            | 0.62 ±0.58                   | 0.59 ±0.22             | 0.325 |
| HGB (g/dl)               | 11.79 ±1.24                  | 11.4 ±1.21             | 0.222 |
| PLT (10³/μl)             | 181.9 ±65.29                 | 188.2 ±47.45           | 0.669 |
| MPV (fl)                 | 11.89 ±0.99                  | 11.5 ±1.14             | 0.173 |
| PLT <100 × 10³/μl        | n= %                         | n= %                   |     |
| WBC, leukocytes; NEU, neutrophils; LYM, lymphocytes; MONO, monocytes; HGB, hemoglobin; PLT, platelets; PDV, platelet volume distribution; MPV, mean platelet volume.

Table 4 Characteristics of new borns

|                          | Preeclampsia group (n = 31) | Control group (n = 30) | P  |
|--------------------------|------------------------------|------------------------|----|
| Weight (g)               | 2555.39 ±795.22             | 3455 ±354.27           | <0.001 |
| Height (cm)              | 48 ±26 – 66                 | 51 ±47 – 54            | <0.001 |
| CAPURRO (WG)             | 38.1 ±0 – 40.6              | 39.7 ±37 – 42.2        | <0.001 |
| APGAR minute 1*          | n= %                        | n= %                   |     |
| Low                      | 3 9.7                       | 1 3.3                  |     |
| APGAR minute 5*          | n= %                        | n= %                   |     |
| Low                      | 2 6.5                       | 1 3.3                  |     |
| New born                 | Alive                       | Death                  |     |
| Live                     | 29 93.5                     | 2 6.5                  | 0.492 |
| Death                    | 2 6.5                       | 0 0                    |     |
| APGAR score (muscle tone, respiratory effort, heart rate, reflex/irritability, and skin color) was given by the pediatrician, with values from 0 to 10 points.
*Values 1 and 5 determine the minutes in which the product was evaluated. Values <7 points in APGAR scores were interpreted as ‘LOW’ otherwise interpreted as ‘PROPER.’
WG = weeks of gestation.
countries, PE is a leading cause of maternal death, accounting for more than 25%.[5] In México, PE is the main cause of death associated with complications of pregnancy.[5]

Iron is abundant in the placenta and participates in the production of free radicals causing oxidative stress, mainly in the second trimester of pregnancy. In situations where the dose of prophylactic iron is exceeded, the proteins to which it binds (ferritin and transferrin) become saturated, leaving this mineral in its free form in the bloodstream, where it becomes one of the principal chemical species involved in producing oxidative stress with potentially damaging effects in both the mother and newborn.[11,13,16] In healthy women, SF concentration below 15–20 μg/l indicates the presence of iron deficiency. Based on this, some authors have proposed to start prophylactic iron when ferritin levels are between 30 and 70 μg/l, while pregnant women with ferritin levels above 70–80 μg/l do not require prophylactic iron.[17] In our study, most patients received iron at the dose recommended by the WHO (60 mg per day) regardless of the SF concentration. In the PE group, 30% of patients had an SF level above 70 μg/l compared with the control group where only 7% of patients were found above this level. Previous studies have found that serum iron is higher in women with PE than in those with a healthy pregnancy.[18,19] We measured and compared the serum concentrations of transferrin, ferritin, and iron in 31 patients with PE and 30 healthy pregnant women, and observed that iron and SF concentrations were higher in the group with PE. The control group had a median SF of 20.9 μg/l while in the PE group the median of ferritin was 36.5 μg/l (P = 0.019). This could be explained by excess exogenous administration of iron, since during the last trimester of a normal pregnancy ferritin levels decrease and remain around 20 μg/l.[20] However, the clinical significance of this statically significant difference in SF concentration between the groups is unclear. In this context, some studies have found that SF concentration ≥ 41 μg/l are associated with a risk of developing preterm birth and infections.[21] Siddiqui et al.[19] found significant higher SF levels in preeclamptic women than in normal pregnant females with a ferritin mean of 32.56 ± 11.72 and 19.89 ± 8.86 μg/l, respectively. Based on this, we performed a sub-analysis taking into consideration those patients with an SF concentration ≥ 40 μg/l, observing that a greater number of women with values ≥40 μg/l were in the PE group (45.2% vs. 20%) (P = 0.05). After Spearman analysis we found that a SF ≥40 μg/l correlated with the presence of PE (r = 0.281; P = 0.032). Ferritin is considered an acute phase response; nevertheless, some studies have demonstrated that an acute phase response is not the principal cause of high ferritin levels in preeclamptic patients.[19]

On other hand, while some studies have found that a red blood cell count, hematocrit and hemoglobin concentrations are higher in women with PE than in healthy pregnancies, others have reported no significant difference.[19] In the present study, we found no statistically significant difference between the groups in these three parameters. Some platelet indices have also been studied trying to predict PE, including the MPV and the PDW.[22] These platelet indices have been evaluated in an attempt to explain the pathophysiology of vascular diseases, including PE, but their value is unknown.[22] In a study including 29 patients with PE, investigators observed that these patients had lower platelet counts than women without PE, suggesting that a platelet count could predict a diagnosis of severe PE. Their results are similar to previous studies, which found lower levels of circulating platelets and a higher MPV in women with PE.[22] We compared the platelet count, MPV and PDW of patients with PE with the control group and found no statistically significant differences (P = 0.669, P = 0.173, and P = 0.470, respectively), this is consistent with previous studies.[22,23] However, analyzing the number of patients with fewer than 100 × 10^3/μl platelets, the incidence of thrombocytopenia was higher in the PE group than in controls (12.9% vs. 3.3%, P = 0.354), similar to that observed in other studies that report an incidence of 15%.[24]

Iron supplementation to pregnant women has been a public health intervention for a long time and there are many studies supporting the benefit of this practice, however, supplementation of pregnant women with iron irrespective of their iron status could put them at risk of unnecessary toxic effects of iron.

**Conclusion**

In this pilot study we found that a higher SF level, despite being within normal range, was associated with PE in pregnant women receiving prophylactic iron. The incidence of thrombocytopenia was higher in preeclamptic women; however, the remaining hematological parameters were similar in both groups. More studies including representative sample sizes are needed to determine the participation of iron in the pathophysiology of PE as well as its proper dosage during pregnancy.

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Conflicts of interest All authors declare that they have no conflicts of interest, and state that this study was performed with funds of the Hematology Service of U.A.N.L. Medical School.

Ethics approval Despite this is an observational study, the protocol was approved by the local ethics committee (Ethics Committee of Medicine School, Universidad Autónoma de Nuevo León, Clave: HE12-006) and all patients signed informed consent form before any procedure.

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