

### Evaluation of the changes in iron homeostasis and hepcidin concentration in preeclamptic patients

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#### Abstract

**Background:** Plasma iron is increased in preeclampsia (PE) in comparison to normal pregnant women. The relation between iron homeostasis and inflammation is hepcidin. Hepcidin is an acute phase reactant protein which has major role in iron hemostasis.

**Objectives:** To evaluate serum iron, total iron binding capacity (TIBC), serum ferritin and serum hepcidin levels in preeclamptic pregnant women in relation to non preeclamptic pregnant women, who were not on any iron supplement regimen.

**Materials and Methods:** This case control study was conducted on twenty pregnant women in the third trimester of pregnancy suffering from preeclampsia that had not received iron supplement or had blood transfusion within last three months, those patients attended the obstetrics and gynecology department at (AL-Imamin AL-Kadhmin medical city) between May to August 2013. Along with those patient twenty non preeclamptic pregnant women who were age and gestational age matched were included as control group. Moreover any subject presented with active infection, chronic diseases, chronic blood loss or twin pregnancy was excluded. A total of 5 ml of venous blood sample was obtained from each patient and control and tested for measurement of Hb, PCV, and MCHC by automated device whereas, serum of iron and TIBC, ferritin and hepcidin were measured by ELISA technique.

**Results:** The mean level of Hb, PCV, MCHC, serum iron, serum ferritin and serum hepcidin in preeclamptic patients were higher than those of control group (P value of < 0.05). There was a non-significant correlation between serum iron and hepcidin in preeclamptic patients ( $r=0.234$ ,  $P=0.32$ ) whereas there was a significant strongly positive correlation between serum iron and hepcidin in the control group ( $r=0.839$ ,  $P=0.003$ ).

**Conclusions:** In preeclamptic patients serum iron concentration is increased in spite of high hepcidin concentration which might indicate a resistance to the iron-decreasing action of hepcidin.

**Keywords:** preeclampsia, hepcidin; pregnancy; iron regulation

## Introduction

Preeclampsia, a systemic syndrome manifested primarily by hypertension and proteinuria, presents mainly in the second half of pregnancy, and affects approximately 3% to 5% of pregnancies worldwide.<sup>(1)</sup>

Normal women has a decrease in serum iron and ferritin during the third trimester of pregnancy as their stores of iron are depleted because of fetoplacental demand and required expansion of red cell mass.<sup>(2,3)</sup>

Plasma iron concentrations and ferritin are increased, whereas total iron binding capacity (TIBC) is decreased in preeclampsia. The increase in plasma iron is in contrast to inflammation characteristic for preeclampsia. The link between iron homeostasis and inflammation is hepcidin.<sup>(4)</sup> Hepcidin, a negative regulator of iron absorption and Recycling<sup>(5)</sup>, is a small peptide produced by the hepatocytes in response to increased body iron and inflammation.<sup>(6)</sup> The increase in plasma iron concentrations despite high hepcidin concentrations in preeclampsia might indicate a resistance to the iron-decreasing action of hepcidin.<sup>(4,7)</sup>

The aims of the study were to evaluate serum iron, total iron binding capacity (TIBC), serum ferritin and serum hepcidin levels in pregnant women suffering from preeclampsia and to compare them with non preeclamptic pregnant women.

## Materials and methods

This study was conducted on twenty preeclamptic pregnant women in the third trimester of pregnancy who were attending the obstetrics and gynecology department at (AL-Imamin AL-Kadhmin medical city) between May to August 2013. Along with twenty non preeclamptic pregnant women served as control and who were age and gestational age matched and both groups had a parity of three or less and twin pregnancy were excluded. All of the preeclamptic patients were diagnosed previously by obstetrical specialist and the mean arterial pressure (MAP) was measured for all pregnant women.

MAP =  $\frac{[(2 \times \text{diastolic blood pressure}) + \text{systolic blood pressure}]}{3}$ .

A written consent for participation in the study was obtained from each subject included in the study.

Moreover pregnant women included in the study were not receiving iron supplement and had not received blood transfusion within the last three months. Also both groups should not suffer from active infection, any chronic disease, and chronic blood loss.

### **Blood sampling:**

A total of 5 ml of venous blood sample was obtained from each patient and control by venipuncture from the cubital fossa under aseptic technique. The blood sample was divided into two smaller samples as follows:

1-First sample comprised of two milliliters of blood in Ethylene diamine tetra acetic acid (EDTA) tube for measurement of Hb, PCV, MCHC by automated haematology analyzer (Sysmex KX-21N, Japan).

2-Second sample comprises of three milliliters of blood in plain tube to obtain serum by centrifugation of clotted blood for measurement of iron and UIBC, ferritin and hepcidin. Serum Iron and UIBC was estimated by the direct colorimetric assay using automated analyzer (ARCHITECT c4000, Abbott, USA) and by commercially available kit using for (IRON, 6K95-30 and 6K95-41, ARCHITECT, Abbott, USA), and for UIBC (UIBC LIQUID, 4P79-30,

ARCHITECT, Abbott, USA). The TIBC was calculated from serum iron concentration plus unsaturated iron binding capacity (UIBC). Ferritin was calculated by enzyme immunoassay based on standard method using automated immunoanalyzer (Minividas, Biomerieux, France) and commercially available kit (VIDAS Ferritin, 30 411, Biomerieux, France). Hepcidin was calculated in patient and control serum by Enzyme linked immunosorbent assay (ELISA) based on biotin double antibody sandwich technology using standard enzyme reader (ELISA Reader, Diagnostic automation inc, USA) and a commercially available kit (Human Hepcidin ELISA kit, MBS164980, MyBioSource, USA).

Computerized statistical analysis were performed using SPSS (statistical package of social sciences), version 16 with Microsoft office excel 2007. The numeric data were expressed as mean $\pm$ SD. Student t-test was used to estimate the difference between two means. Correlation test was used to find the association between two numerical variables.

## Results

This study included twenty preeclamptic pregnant women in third trimester of pregnancy with mean age was  $28.5 \pm 4.9$  years. Along with twenty non preeclamptic pregnant women who were age and gestational age matched and who served as control group, whose mean age was  $28.3 \pm 6.3$  years. The mean arterial pressure was significantly higher in preeclamptic patient ( $114.9 \pm 8.9$  mmHg ) than the mean arterial pressure control group ( $87.8 \pm 6.2$  mmHg) Haematological and biochemical parameters of preeclamptic patients and control group were presented in Table 1, showing that the mean level of Hb,PCV,MCHC as well as mean level of serum iron, serum ferritin, serum hepcidin of preeclamptic patients were significantly higher than those of

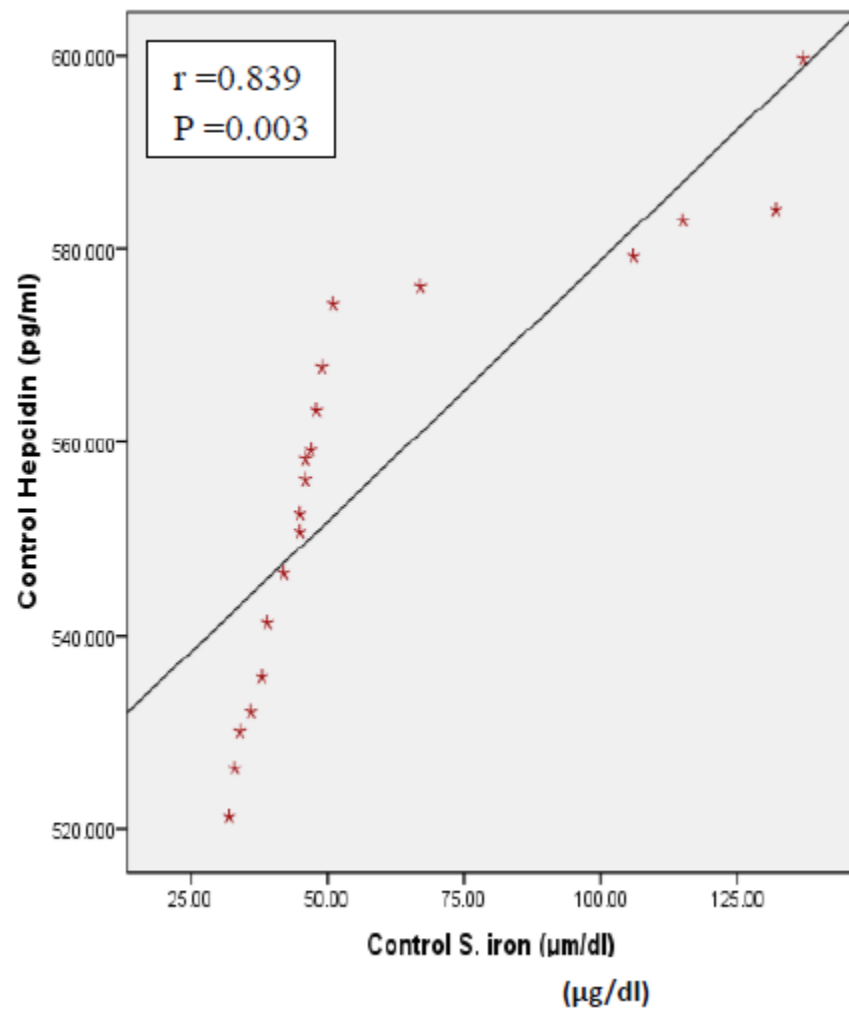
control group. Only the mean level of TIBC was lower in preeclamptic patients than control group.

Furthermore , there was a non significant correlation between serum iron and hepcidin in preeclamptic patients ( $r=0.234$ ,  $P= 0.32$ ) whereas this correlation was strongly positive in normal pregnant control group ( $r=0.839$ ,  $P = 0.003$ ). (Figure 1)

**Table 1:** Haematological and biochemical parameters of preeclamptic patients and control group

	Preeclamptic patients (Mean±SD)	control group (Mean±SD)	P value
Hb (g/dl)	12.142±1.9	9.9±2.1	0.004*
PCV %	37.63±5	33.31±5.7	0.012*
MCHC (g/dl)	32.365±1.5	29.47±2.4	0.004*
Serum iron (µg/dl)	111.1±27.2	59.4±33.8	0.003*
TIBC (µg/dl)	402.65±86.9	484.95±97.5	0.012*
Serum ferritin (ng/ml)	24.78±6.9	12.3±6.5	0.001*
Serum hepcidin (pg/ml)	797.38±249.1	556.873±21.8	0.004*

\*Significant P value(&lt;0.05)



**Figure 1.** Correlation between serum iron and hepcidin in control group ( $P = 0.003$ )

## Discussion

The present study had revealed that iron profile (includes S.iron, TIBC, S.ferritin was within normal range in preeclamptic patient although they were not receiving any iron supplement. Whereas the iron profile in normal pregnancy women was that of iron deficiency anemia, which was expected since they were not receiving iron supplements. Those results were in agreement with studies done by Tasneem zafar et al <sup>(3)</sup>, Gergely Toldi et al <sup>(4)</sup> and Margaret P. Rayman et al<sup>(8)</sup>. These results may be attributed to that, in preeclampsia there is impairment of trophoblast invasion to the maternal spiral arteries resulting in necrotic and hemorrhagic areas in the placental tissue. The injured red blood cells in those area will be a source of free iron radical. <sup>(8,9)</sup>

Additionally, Margaret P. Rayman et al in their study had found that, iron released from red-cell destruction of damaged placenta are clearly capable of initiating and propagating lipid peroxidation, results in endothelial-cell damage in preeclamptic patients.<sup>(8)</sup> So we may propose that disturbance of iron status is a cause and result for preeclamptic changes. Consequently, preeclamptic patients had normal Hb, PCV and MCHC levels, whereas

control group had low Hb, PCV and MCHC levels since those patients had not received iron supplement. Those results were in agreement with studies done by Tasneem zafar et al.<sup>(3,10)</sup>

This study showed that, the mean level of serum hepcidin in preeclamptic patients was within normal range and significantly higher than that control group and there was no correlation of its level with S. iron. This result was in agreement with study done by Gergely Toldi et al, who found that increased in the pro-inflammatory cytokine; interleukin-6 (IL-6) in preeclamptic patients as a result of inflammatory reaction, results in high hepcidin concentration in those patients.<sup>(4)</sup> despite of high S. iron level which might indicate a resistance to the iron-decreasing action of hepcidin. This resistance to hepcidin may be due to functional or structural abnormalities of ferroportin. It is well known that normally the chronic inflammation decreases iron availability through decreasing the release of iron from the macrophages to the plasma as a result of raised serum hepcidin <sup>(11)</sup>, but this drive is absent in preeclamptic patients.<sup>(4,7)</sup> Thus we may propose that in preeclamptic patients the ongoing inflammation had no

effect on iron status through hepcidin.<sup>(7,12)</sup>

Where as in normal pregnant women hepcidin level was significantly decrease in relation to low S. iron level .

## **Conclusion**

In preeclamptic patients serum iron concentration is increased despite of high hepcidin concentration thus we may suggest that pregnant women not receiving iron therapy and even more had normal Hb , high S.iron ,and high hepcidin may suggest an impeding preeclampsia , close follow up is recommended , thus for these pregnant women iron status should be assessed before giving iron supplement.



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## تقييم التغيرات في تركيز الهبسيدين وتوازن الحديد عند الحوامل المصابات بتسمم الحمل

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### الملخص

**الخلفية:** يتم زيادة بلازما الحديد في تسمم الحمل بالمقارنة مع النساء الحوامل الطبيعيين و العلاقة بين توازن الحديد والالتهاب هي هيبسيدين. هيبسيدين هو بروتين تفاعلي حاد المرحلة الذي له دور كبير في توازن الحديد. **الأهداف:** تقييم مستويات حديد المصل، ومجموع الحديد ملزم قدرة ، مصل الفيريتين وهيبسيدين المصل في النساء الحوامل المصابات بتسمم الحمل فيما يتعلق بالنساء الحوامل الاصحاء، الذين لم يكونوا على أي نظام علاجي للحديد. **المواد والطرق:** أجريت هذه الدراسة على عشرين من النساء الحوامل في الثلث الثالث من الحمل اللواتي يعانين من تسمم الحمل و لم يعطين الحديد و لم يتم نقل الدم لهم في الأشهر الثلاثة الأخيرة، هؤلاء الحوامل حضرن الى قسم أمراض النساء والتوليد في مستشفى الامامين الكاظمين بين مايو-أغسطس عام 2013. وإلى جانب هؤلاء المرضى عشرين امرأة حامل غير مصابات بتسمم الحمل الذين كانوا في نفس العمر والعمر الحولي وأدرجت كمجموعة مراقبة مطابقة. وعلاوة على ذلك تم استبعاد أي حامل مع عدوى نشطة، او لديها أمراض مزمنة، اوفقدان الدم المزمن أو الحمل التوأم. تم الحصول على ما مجموعه 5 مل من عينة من الدم الوريدي من كل مريض ومراقبة واختبار لقياس الهيموجلوبين، PCV، و MCHC بواسطة جهاز آلي في حين تم قياس مصل الحديد TIBC، الفيريتين وهيبسيدين بواسطة تقنية ELISA.

**النتائج:** كان متوسط مستوى الهيموجلوبين، PCV، MCHC، حديد المصل، الفيريتين في المصل وهيبسيدين المصل في المرضى الذين يعانون تسمم الحمل أعلى من تلك التي المجموعة الضابطة (قيمة P من  $0.05 >$ ) كان. هناك علاقة غير ذات دلالة إحصائية بين الحديد في الدم وهيبسيدين في المرضى الذين يعانون تسمم الحمل ( $r = 0.234, P = 0.32$ ) في حين كان هناك ارتباط إيجابي قوي كبير بين الحديد في الدم وهيبسيدين في السيطرة على المجموعة ( $r = 0.839, P = 0.003$ ).

**الاستنتاجات:** كان الحوامل مع تسمم الحمل لديهم زيادة تركيز الحديد في الدم على الرغم من تركيز هيبسيدين العالية التي قد تشير إلى وجود مقاومة للعمل على خفض الحديد من هيبسيدين. **الكلمات المفتاحية:** تسمم الحمل، هيبسيدين. حمل؛ تنظيم الحديد