Correlative study of serum hepcidin levels and serum iron reserve parameters in preeclampsia and HELLP syndrome

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Received 20 March 2018; revised 26 June 2018

HELLP syndrome is described as a special group of severe preeclampsia characterized by haemolysis (H), elevated liver enzymes (EL) and low platelets (PL), whereas preeclampsia is a disease of unknown etiology characterized by hypertension and proteinuria after the 20th week of gestation. The objective of the study was to evaluate the level of hepcidin in the serum of the patient suffering from HELLP syndrome and preeclampsia. It was done by competitive sandwich ELISA which assesses the serum level of hepcidin in the forty patients suffering from preeclampsia and HELLP syndrome (as cases) and forty healthy individuals (as control). The results of our study showed that serum hepcidin levels were significantly increased among the cases ($P=0.00171$) in comparison to control ($P<0.05$) and transferrin saturation levels were significantly decreased ($P<0.05$) among the cases ($P=0.001$) in comparison to controls. The decrease in transferrin saturation indicates that there is low serum iron level among the cases. It was concluded that increased hepcidin level may be the cause of anemia among the cases.

Keywords: ELISA, HELLP syndrome, Hepcidin-25, Preeclampsia, Transferrin

Pregnancy may lead to various complications such as gestational diabetes, proteinuria, hypertension preeclampsia, eclampsia etc. Hypertensive disorders during pregnancy are major causes of morbidity and mortality. In the developing world, it leads to 10-15% of maternal deaths¹. It may complicate 3-10% pregnancies depending on different hospitals and countries². It is classified into four groups, chronic hypertension, gestational hypertension, preeclampsia and eclampsia (Report of the National High Blood Pressure Education Program, 2000). Preeclampsia is a disease of unknown etiology characterized by hypertension and proteinuria after the 20th week of gestation².

A special group of severe preeclampsia patients are characterized by some special features and called as HELLP syndrome³. HELLP syndrome is characterized by hemolysis (H), elevated liver enzymes (EL) and low platelets (LP). The incidence of HELLP syndrome is 0.2-0.8%⁴. Although various theories have been proposed the exact pathogenesis is not known. Among all hypertensive disorders of pregnancy, HELLP syndrome bears the worst prognosis with maternofetal complications of 7-70% and maternal mortality rate of 1-24%⁴. Hepcidin was first isolated from human urine and named on the basis of its site of synthesis (hep-) and its in-vitro antibacterial properties (-cidin). The primary site for hepcidin production and secretion is liver which is also thought to be involved in sensing iron circulation and iron store⁵,⁷. The production of hepcidin can be assessed by measuring the level of hepcidin peptide in serum (human)⁸. Hepcidin can also be measured in urine as peptide is also excreted through the kidney.

In order to describe the postulated major role of hepcidin, it is necessary to understand the function of ferroportin which is located on tissues that actively export iron including intestinal enterocytes, reticuloendothelial macrophages, and hepatocytes. The efflux of iron into plasma is controlled by hepcidin by regulating Ferroportin. Hepcidin directly binds to ferroportin and decreases its functional activity by causing it to be internalized from the cell surface and degraded⁹. The production of hepcidin by the liver is simultaneously regulated by circulating and stored iron, erythropoietic activity, and inflammation⁸. Therefore, at any time, the expression of hepcidin is determined by the interplay of these pathways and the relative strength of each of the

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individual signals\textsuperscript{10}. When there is any kind of inflammation or infection is present and the body iron level is elevated liver hepcidin production is increased resulting in diminished Ferroportin expression. Conversely, when anemia or hypoxia exists and body iron level reduced, hepcidin expression is also reduced, allowing for increased dietary iron absorption and mobilization from body stores via active Ferroportin. Furthermore, iron absorption is markedly influence by hepcidin concentration and can affect the efficacy of iron repletion via supplemental or dietary sources\textsuperscript{11}. Therefore hepcidin regulates the systemic iron availability and is critical throughout pregnancy, it is important to evaluate hepcidin concentrations in both uncomplicated and complicated pregnancies and throughout gestation.

The objective of the study was to investigate the serum hepcidin level and serum transferrin level in the patients of PE and HELLP syndrome and to examine the relationship between serum hepcidin level and iron parameters in PE and HELLP syndrome. Thus, we conducted a prospective case-control study in which we assessed serum hepcidin and transferrin level to observe if there is any correlation between these two parameters.

Materials and Methods
The present study was conducted in the Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi in collaboration with Department of Obstetrics and Gynecology & Department of General Medicine during the period of December 2015 and October 2017. After considering the mean and standard deviation of the diseases Preeclampsia and HELLP syndrome forty cases and forty controls were taken for the study. The diagnosed cases of preeclampsia and HELLP syndrome were selected from the Department of Obstetrics & Gynecology and Department of Medicine of Sir Sunderlal Hospital, Banaras Hindu University. Forty healthy women matched for age were included for comparison as controls. For this study ethical clearance was taken from Institute ethical committee. Informed consents were taken both from cases and controls.

Criteria for selection

Inclusion Criteria for cases
Forty diagnosed cases of Preeclampsia and HELLP syndrome were taken. The diagnostic criteria for preeclampsia were pregnant patients with more than 20 weeks of pregnancy and systolic blood pressure above 140 mm Hg or diastolic pressure above 90 mm Hg & presence of proteinuria of greater than or equal to 0.3 g in a 24 h urine specimen. For HELLP syndrome pregnant cases with laboratory evidence hemolysis increased liver enzymes (liver transaminase ALT/AST levels two times the upper limit of normal), low platelets count of 5000/mL were taken.

Selection of controls
Forty normotensive with no evidence of proteinuria, hemolysis, elevated liver enzymes or low platelets count and pregnancy above 20 weeks were taken for study.

Exclusion criteria for cases and controls
Case selection excluded cases with chronic diseases such as gestational diabetes, tuberculosis, rheumatoid arthritis, hemochromatosis with comorbidity.

Sample collection
Blood samples were collected from patients of preeclampsia and HELLP syndrome. The period of sample collection was from December 2015 to October 2017. Taking all aseptic precautions, about 5 mL of blood was drawn by venipuncture from a peripheral vein, with a disposable syringe. The blood thus collected in clean dry glass tubes were allowed to stand for 30 min at room temperature for the retraction of the clot. Then the samples were centrifuged at 3000 rpm for 10 min to separate the serum. The serum samples were stored at −20°C until assayed.

Estimation of serum hepcidin
The ELISA kit [Catalog No: E-EL-H0077/Lot: AK0017APR01042] for hepcidin used competitive Sandwich-ELISA method. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to Human Hepcidin-25. Standards or samples were added to the appropriate micro ELISA plate wells and bound by the specific antibody. Then a biotinylated detection antibody specific for human hepcidin-25 and avidin-horseradish peroxidase (HRP) conjugate was added to each microplate well successively and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contain human hepcidin-25, biotinylated detection antibody and avidin-HRP conjugate were appeared
blue in color. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm (Table 1). The OD value was proportional to the concentration of human hepcidin-25. The concentration of human hepcidin-25 in the samples was determined by comparing the OD of the samples to the standard curve (Fig. 1). Optical density is directly proportional to the concentration of hepcidine serum samples. Optical Density was measured to assess the concentration of hepcidin in the cases and controls.

**Estimation of Serum Transferrin**

Serum transferrin level was done using an immunoturbidity method by fully automated analyzer after estimation of serum iron and total iron binding capacity.

**Statistical Analysis**

All data analysis was done using R language and R studio software. Two-tailed test was performed on the concentration level of hepcidin (Fig. 2). Mean and standard deviation was calculated and the two groups were analyzed using Welch’s independent two sampled t-test for equality of means.

**Results**

The important characteristics of the study are shown in (Table 1) (prospective). In the study, it was observed that serum hepcidin levels were significantly increased ($P=0.00171$) among cases in comparison to controls ($P<0.05$) and serum transferrin levels were significantly decreased ($P<0.05$) among cases in comparison to controls.

The correlation co-efficient between hepcidin & transferrin is $-0.2792887$. This correlation co-efficient is statistically significant. However, it suggests a mild negative association between hepcidin & transferrin. So, an increase in hepcidin concentration would lead to the decrease in the concentration of transferrin scatter plot of hepcidin & transferrin also indicates a mild association. A linear curve was fitted with hepcidin as predictor and transferrin as response as shown in (Fig. 3). The fit statistic $r^2=0.078$ suggesting that the line is able to explain 7.8% variability in transferrin. The relationship is captured by the equation: $y = 17 - 0.68x$ (Fig. 4).

**Table 1 — Characteristics of study population in second trimester of pregnancy (prospective)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control (n =40)</th>
<th>Cases (n =40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>33.85 (4.63)</td>
<td>30.7 (3.03)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>33.13 (6.13)</td>
<td>31.6 (2.08)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>104.83 (8.06)</td>
<td>160.56 (13.20)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>74.03 (7.10)</td>
<td>105.20 (7.07)</td>
</tr>
<tr>
<td>Proteinuria (g/24 h min)</td>
<td></td>
<td>4.86 (2.9)</td>
</tr>
</tbody>
</table>

**Fig. 1 — Shows concentration versus optical density of hepcidin standards**

**Fig. 2 — Mean and standard deviation of hepcidin level**

**Fig. 3 — Mean and standard deviation of serum transferrin level**
This means that hepcidin alone cannot explain the variance in transferrin and is not a strong predictor for the same.

Discussion

Our result confirmed the initial hypothesis that hepcidin level is increased in PE and HELLP syndrome and there is a negative correlation between serum hepcidin and transferrin level.

After quantitative estimation, the data was analyzed using statistics and found the mean and standard deviation of serum hepcidin levels for cases 5.773 and 3.442, and for controls 3.560 and 2.46, respectively. Statistical analysis showed that the means are not from the same group with P-value [0.00717]. From the data, it was concluded that there was a significant increase in hepcidin levels among HELLP syndrome and preeclampsia patients in comparison to controls. Serum transferrin levels were significantly lower in cases as compared to controls (P <0.05). Lower serum transferrin levels indicate iron deficiency anemia among cases. For lower serum transferrin levels it can hypothesize that it is due to the increased hepcidin-induced internalization of ferroportin. We can say that Hepcidin is a negative regulator of iron transport into plasma that acts by binding ferroportin (the only known cellular iron exporter) thus leading to ferroportin degradation. The main cell targets of hepatic hepcidin are duodenal enterocytes and macrophages, where it regulates dietary iron absorption and inhibits the release of iron derived from senescent erythrocytes.

So from this study, it can be hypothesized that there is a significantly higher level of hepcidin among preeclamptic and HELLP syndrome patients and there is a significantly decrease in iron reserves among cases.

Conclusion

Our study adds key new information on serum hepcidin concentration in preeclampsia and HELLP syndrome patients. For the first time to our knowledge, a prospective study was performed in order to verify if there is any correlation between hepcidin serum levels and serum transferrin level. We obtained significant and encouraging results. High hepcidin maternal serum levels could be an early marker of PE.

References