Influence of hepcidin on iron homeostasis during last trimester of gestation in 
*Bos indicus* (cattle)

K. Rajamanickam*, M. Sameer Ali and V. Leela

Department of Veterinary Physiology,
Madras Veterinary College, TANUVAS, Chennai-600 007, Tamil Nadu, India.

Received: 17-10-2018 Accepted: 04-02-2019 DOI: 10.18805/ijar.B-3743

**ABSTRACT**

Hepcidin is an important hormone regulating the systemic iron bioavailability. Blood samples were collected from thirty pregnant cattle during their last trimester of pregnancy to assess the relation of hepcidin to iron homeostasis. Serum hepcidin level was quantified using ELISA and serum iron, transferrin iron binding capacity and unsaturated iron binding capacity were estimated by colorimetric method. Correlation between hepcidin and other iron related parameters was assessed. Dependency of serum iron level on hepcidin was also determined by regression method. It was revealed that hepcidin was negatively correlated to serum iron and transferrin iron binding capacity (p< 0.001) and also hepcidin has highest predictive value on serum iron level and transferrin iron binding capacity (p< 0.001). It can be concluded that during pregnancy increase in hepcidin reduces the maternal serum iron and also it is a biomarker for iron bioavailability to the developing fetus.

**Key words:** Bioavailability, Cattle, Hepcidin, Pregnancy, Serum iron.

**INTRODUCTION**

Iron is an important micro-mineral playing a vital role in many metabolic processes. It is essential for oxygen transport and regulation of cell growth and differentiation in mammals (Pantopoulos et al. 2012). During pregnancy, fetal and placental growths are supported by iron and it also increases the maternal blood cell mass in later stages of gestation to compensate the blood loss during parturition (Scott, 1972). Iron deficiency during pregnancy will cause anaemia, hypoxia and dysfunction of myocytes, epithelia and nervous system(Greer, 2009). In recent years many new molecules have been identified as a regulator of systemic iron homeostasis, among them hepcidin has a central role. Hepcidin is a peptide hormone synthesised mainly from the liver, which regulates efflux of iron from the cell into plasma by internalization and ubiquitination of ferroportin molecule. Expression of hepcidin mRNA in the liver mainly depends on serum iron level (Rajamanickam et al. 2017). In veterinary medicine, presence of hepcidin expression was detected in cattle, dog, cat, pig, goat, etc., (Hilton and Lambert, 2008). Unlike other peptides hepcidin sequences are similar among various mammalian species (Khangembam and Kumar, 2011). Apart from liver, expression of hepcidin is also identified in bovine placental tissues, which indicates the importance of hepcidin in iron homeostasis during pregnancy (Roperto et al. 2017). Therefore, the serum iron availability is important during pregnancy for both dam and developing fetus, and hepcidin regulates systemic bioavailability of iron. It is important to assess the hepcidin concentration during third trimester of pregnancy in cattle as rapid development of fetus occurs. Hence this research work was carried out to identify the concentrations and relationship between hepcidin and serum iron in pregnant cattle during third trimester of gestation.

**MATERIALS AND METHODS**

Blood samples were collected from thirty pregnant dairy cattle during their third trimester of pregnancy (7 to 9 months of gestation) from organized dairy cattle farm, reared in intensive system of management and fed with ration of roughage and concentrate in the ratio of 2:1. Majority of these animals were in fourth parity (average gestation period was about 240±10 days) and had body condition score more than 3.5 to 5. All these animals gave birth to healthy calves, none of them had pregnancy complications. Serum was immediately separated and stored in -80°C for analysis. Concentration of hepcidin was assessed by using sandwich ELISA kit supplied by Sincere Biotech, Beijing101300, China. Serum iron, transferrin iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were estimated from all the samples by Ferrozine method using colorimetric kit supplied by Coral clinical systems, Goa, India 403202. Data obtained were subjected to analysis as per the methods explained by Snedecor and Cochran. (1994). The concentration of hepcidin, serum iron, TIBC and UIBC were represented as mean± standard error of the mean. Pearson correlation was used to identify the type of relationship...
among hepcidin, serum iron, TIBC and UIBC. Linear regression model was used to identify degree of dependency of serum iron, TIBC and UIBC to the hepcidin hormone. All analyses were performed using SPSS IBM Version 23 software. All the graphs were constructed using GraphPad PRISM software.

RESULTS AND DISCUSSION

The serum iron level in the pregnant cattle during their third trimester was found to be 193.00±24.39 µg/dL with range of 101.30 µg/dL to 362.50 µg/dL. Transferrin iron binding capacity of these cattle was 274.15±28.88 µg/dL with range of 138.70 µg/dL to 401.61 µg/dL. Unsaturated iron binding capacity of these cattle was 70.64±8.71 µg/dL with range of 35.20 µg/dL to 111.60 µg/dL. Hepcidin concentration of the pregnant animals was about 36.08±4.14 ng/mL with range of 15.89 ng/mL to 67.38 ng/mL (Fig 1).

The serum iron concentration of these animals was found to be higher than the normal iron concentration (97±29 µg/dL) of non-pregnant cattle (Kaneko et al. 1997). Similar result was obtained by Djokovic et al. (2014) in cows during their late gestational period. During pregnancy to increase the rate of haemoglobin synthesis for oxygenating the tissues to sustain the rapid fetal growth there will be accelerated demand for iron (Cogswell et al. 2003). This resulted in the increased serum iron in maternal blood.

Maternal iron enters the fetal circulation through initiation of transferrin cycle by the formation of clathrin-mediated endosomes in the placental epithelial cells. Initiation of the transferrin cycle occurs only when the serum iron binds with apo-transferrin produced from the liver. Hence during pregnancy expression of transferrin molecule will also increase to mediate the above said process (Harris, 1992). This explains the increase in transferrin iron binding capacity of these animals than the TIBC (230±65 µg/dL) of non-pregnant animals (Kaneko et al. 1997). Unsaturated iron binding capacity of these animals was lower than the UIBC (131±36 µg/dL) of non-pregnant animals (Kaneko et al. 1997). This reveals most of the serum iron was in the bound form with transferrin, which was required to transport iron for the developing fetus across placental tissue.

On assessing the relationship between hepcidin and iron related parameters by Pearson correlation, it was revealed that hepcidin was negatively correlated to serum iron and transferrin iron binding capacity at the level of p<0.001 significance (Table 1). Hepcidin was not correlated to unsaturated iron binding capacity of these animals.

This indicates animals having higher serum hepcidin concentration will have lower systemic iron level. When hepcidin concentration increases it binds with hepcidin binding domine of ferroportin molecule leading to its JAK2-dependent tyrosine phosphorylation and degradation. Since ferroportin is the sole exporter of iron from intracellular compartment to systemic circulation, degradation of this molecule decreases the systemic iron level (De Domenico et al. 2009). This was proved by overexpressing hepcidin during embryonic development of transgenic mice resulted in spontaneous abortion in utero due to severe iron deficiency (Nicolas et al. 2002).

As a result of increased hepcidin level there will be reduced availability of serum iron for transferrin binding, this was reflected as negative correlation between hepcidin and transferrin iron binding capacity in the these animals. Maternal hepcidin regulates the iron-transferrin complex formation by dictating the iron flux coming from dietary intake and storage sites. Therefore, maternal hepcidin determines the availability of iron for placental uptake (Nemeth and Ganz, 2006).

![Fig 1: Serum hepcidin, iron, transferrin iron binding capacity and unsaturated iron binding capacity of pregnant cattle with mean and standard error of the mean.](image1)

![Fig 2: Relation between hepcidin and serum iron with regression equation and r² value.](image2)

![Fig 3: Relation between hepcidin and transferrin iron binding capacity (TIBC) with regression equation and r² value.](image3)
**Table 1**: Correlation of hepcidin with serum iron, TIBC and UIBC in pregnant cattle.

<table>
<thead>
<tr>
<th>Pearson correlation</th>
<th>Serum iron</th>
<th>TIBC</th>
<th>UIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin</td>
<td>Correlation coefficient</td>
<td>-0.902**</td>
<td>-0.914**</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.0002</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

**Significance at p<0.001**

Linear regression model revealed that the concentration of serum iron was highly dependent on hepcidin concentration with r square value of 0.8127 and it was significant at p<0.001 (Fig 2). Hepcidin also influences the transferrin iron binding capacity of these animals with r square value of 0.836 at the level of p<0.001 significance (Fig 3).

During pregnancy depending upon the iron demand by dam and fetus, hepcidin alters the circulating iron level by acting on iron exportation molecule (Blackburn, 2003). An experiment in pregnant rodents also proved that to compensate increased iron demand expression of hepcidin mRNA was 1.9% of non-pregnant values and returned to normal concentration within 24 to 48 h postpartum (p<0.05) (Millard et al. 2004). In the present study, pregnant animals with lower hepcidin concentration had highest serum iron level. This proves hepcidin has major influence on iron homeostasis during pregnancy.

Apart from maternal hepcidin, fetal hepcidin also has greater influence on determining the degree of iron transfer through placental tissue. Fetal hepcidin regulates the entry of iron into fetus by controlling the expression of ferroportin molecule on placental tissues which has contact with fetal blood circulation (Nemeth and Ganz, 2006). Thus, both maternal and fetal hepcidin has greater influence on iron homeostasis during pregnancy.

Upto authors knowledge this was the first work which quantifies the serum hepcidin level in pregnant *Bos indicus*(cattle). In the current study, animals on third trimester of pregnancy and mainly on fourth parity were selected. Further research can be done in both fetus and dam with different stages of pregnancy and parities using various quantification techniques to identify the direct and indirect effect of hepcidin on iron homeostasis.

**CONCLUSION**

Hepcidin is an iron regulating hormone and can be used as a biomarker in determining the serum iron bioavailability during pregnancy in both dam and fetus. Since, hepcidin was strongly correlated to serum iron and transferrin iron binding capacity, variations in hepcidin level during pregnancy can help to identify iron related disorders and thereby pregnancy complications can be avoided. Research on hepcidin level during different stages of pregnancy can be used as a diagnostic indicator of maternal iron bioavailability in species where the iron deficiency anaemia is a common condition in both dam and fetus.

**REFERENCES**


