Relationship between hepcidin and oxidant/antioxidant status in calves with suspected neonatal septicemia

E. E. Erkilici, H. M. Erdogani, M. Oguni, A. H. Kirmiziguli, E. Gokcei, M. Kuru1 and A. Kukurt2

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Kafkas, 36100, Kars, Turkey; 2. Department of Biochemistry, Faculty of Veterinary Medicine, University of Kafkas, 36100, Kars, Turkey; 3. Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Kafkas, 36100, Kars, Turkey.

Corresponding author: E. E. Erkilic, e-mail: ekin_emre_24@hotmail.com, HME: hmerdogan@hotmail.com, MO: metinogun@hotmail.com, AHK: ahkirmizigul@hotmail.com, EG: erhangokce36@hotmail.com, MK: mushapkuru@hotmail.com, AK: samedkukurt@gmail.com

doi: 10.14202/vetworld.2016.1238-1241 How to cite this article: Erkilic EE, Erdogan HM, Ogun M, Kirmizigul AH, Gokce E, Kuru M, Kukurt A (2016) Relationship between hepcidin and antioxidant status in calves with suspected neonatal septicemia, Veterinary World, 9(11): 1238-1241.

Abstract

Aim: This study has been conducted for the purpose of determining serum hepcidin, total antioxidant status (TAS), total oxidant status (TOS), and Fe levels in calves with suspected neonatal septicemia before and after treatment and the clinical significance of hepcidin in calves with suspected neonatal septicemia.

Materials and Methods: The study material consisted of 15 calves of different ages and sexes brought to the Training, Research and Application Center at the Kafkas University Faculty of Veterinary Medicine with suspected neonatal septicemia. 8.5 mL of blood was drawn from the jugular vein of each animal into coagulant tubes before and after treatment for one-off biochemical analyses and centrifuged. After this, the serum was separated. Hepcidin, TAS, TOS, and Fe levels in the serum were measured.

Results: While pre-treatment hepcidin levels were 58.42±3.46 ng/mL, post-treatment levels were 46.87±2.98 ng/mL (p<0.05). Pre-treatment Fe levels were 60.13±7.27 μg/dl, while post-treatment levels were 83.1±8.09 μg/dl (p<0.05). The changes in the TAS and TOS levels were also found to be statistically significant.

Conclusion: In light of the fact that hepcidin plays a role function in the regulation of Fe as well as the fact that Fe is a significant nutritional source for many microorganisms, it was concluded that hepcidin may play a significant role in nutritional immunity and the pathogenesis of diseases.

Keywords: Fe, hepcidin, oxidative stress, septicemia.

Introduction

Neonatal calf septicemia causes high morbidity and mortality and is one of the leading and most significant difficulties in raising cattle. Calf septicemia is the main cause of death in the neonatal period [1]. Its etiology involves bacteria (commonly Escherichia coli), viruses (rota and coronavirus), parasites, and other factors. As the disease progresses quickly and is lethal, diagnosis and treatment should be initiated as quickly as possible [2].

Hepcidin is a low molecular weight, antimicrobial peptide hormone and was first discovered in human urine [3]. It is produced by the liver as a first-line response to inflammatory reactions and high Fe concentrations [4,5]. Hepcidin plays a fundamental role in the regulation of Fe metabolism [6], which is a part of foundational cellular functions and thus of vital importance. On the other hand, by participating in redox reactions leading to the production of reactive oxygen species (ROSs), Fe also causes oxidative stress. Therefore, Fe has been regarded as a potential toxic element to cells [7]. Fe also plays an important role in pathogenesis of bacterial infections as bacteria utilize Fe for survival, growth and proliferation; therefore, it is of paramount importance to control the Fe metabolism [6]. It is well known that the abundance of Fe suppresses defense system leading host vulnerable to infections. There is a significant relationship between Hepcidin, Fe metabolism, inflammation, and the immune system. The fact that hepcidin plays an active role in the regulation of Fe release from macrophages and in the control of excessive Fe absorption from the duodenum is well documented [6]. Hepcidin is a part of the natural defense mechanism, thus it limits the amount of Fe that can be utilized by pathogens [8]. In inflammatory conditions, hypoferremia is an important first-line protective mechanism in response to infections [9]. Fe also participates in redox reactions, causing the production of ROS, and thus leading to oxidative stress [7]. Free radicals play a significant role in the pathogenesis of many diseases [10]. Newborns are subject to oxidative stress during birth. It is also reported that in livestock
diseases, especially enteritis and pneumonia, antioxidant capacity is efficacious [11].

This study was designed to determine the clinical significance of hepcidin in calves with suspected neonatal septicemia by evaluating serum hepcidin, total antioxidant status (TAS), total oxidant status (TOS), and Fe levels in calves suspected of neonatal septicemia before and after treatment.

**Materials and Methods**

**Ethical approval**

This study was conducted after obtaining approval from the Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (MAKU-HADYEK-Submission: 2014/77).

**Animals**

The study consisted of 15 calves with suspected neonatal septicemia aged between 1 and 10 days old admitted to the Teaching Hospital of Veterinary Medicine. Suspected septicemia was diagnosed based on clinical (diarrhea, weakness in or absence of sucking reflex, the calf being in a supine position on the ground or being unable to stand, severe dehydration, abnormal rectal temperature [hypo- or hyperthermia], mucosal hyperemia, and full sclera) and hematological (increase in white blood cell [WBC] count) examinations; the animals were suspected to have septicemia [12,13]. The animals were given standard treatment (antibiotic, nonsteroidal anti-inflammatory drugs, vitamin C, fluid therapy, and intestinal astringent). For determination of serum hepcidin, TAS, TOS, Fe levels, and hematological parameters; blood samples were taken before and after treatment in all cases. 8.5 mL of blood was taken from the jugular vein of each animal into coagulant tubes for biochemical analysis and 3 mL blood was taken into EDTA tubes for hematological analysis. Samples were centrifuged at 3000 rpm for 10 min, and the serum was harvested and kept at −20°C until the analysis. Serum hepcidin (Mybiosource®, TAS (Rel Assay Diagnostics®), and TOS (Rel Assay Diagnostics®) were determined using commercial ELISA kits, and Fe value was measured spectrophotometrically. Hematological (WBC, lymphocyte [LYM], red blood cells [RBC], mean corpuscular volume (MCV), and hematocrit [HCT]) analysis was performed on blood counter (VG-MS4e®, Melet Schloesing, France).

**Statistical analysis**

The results were evaluated using the t-test in the SPSS® (SPSS 20, USA) statistical package program to determine the differences between values before and after treatment.

**Results**

**Clinical examination findings**

Calves with suspected septicemia exhibited clinical signs of loss of appetite, fatigue, indifference to surroundings, reduced/absence of sucking reflex, cool extremities, inability to stand, diarrhea, eye sinking into their sockets, and hyperemia in the conjunctiva. The average body temperature, heart rate, and respiratory rates of the animals were 37.18±0.13°C, 104±4.33/min, and 28.86±0.75/min pre-treatment; and 38.54±0.1°C, 107.53±2.20/min and 26.40±0.36/min post-treatment, respectively.

**Biochemical findings**

The changes in hepcidin, TAS, TOS and Fe levels in the calves with suspected septicaemia before and after treatment are given in Table-1. After treatment, serum hepcidin and TOS levels were significantly lower than before treatment in calves. On contrary, serum TAS and Fe levels were significantly higher than before treatment (Table-1).

**Hematological findings**

The treatment of calves resulted in significant changes in the hematological parameters that were examined except for RBC. The WBC count, LYM count, MCV and HCT significantly changed after treatment when compared to values obtained before treatment (Table-2).

**Discussion**

This study aimed to determine the clinical importance or use of hepcidin by comparing the values of serum hepcidin, TAS, TOS and Fe levels in calves with suspected neonatal septicemia before and after treatment.

Clinicians rely on clinical and laboratory examinations of patients to form a working diagnosis, so hematological and serum biochemical parameters are usually used for this purpose [14]. The hematological parameters (WBC, HCT, LYM, and MCV) evaluated in this study were comparable with those reported by

**Table-1:** Serum biochemical changes (X±SE) in calves before and after treatment (n=15).

| Parameters                  | Before treatment | After treatment | p     |
|----------------------------|------------------|----------------|-------|
| Hepcidin ng/mL             | 58.42±3.46       | 46.87±2.98     | p<0.05|
| TAS mmol Trolox equivalent/L| 0.50±0.02        | 0.58±0.03      | p<0.05|
| TOS mM H$_2$O$_2$ equivalent/L| 5.30±0.74        | 2.13±0.2       | p<0.05|
| Fe μg/dL                   | 60.13±7.27       | 83.1±8.09      | p<0.05|

SE=Standard error, TAS=Total antioxidant status, TOS=Total oxidant status

**Table-2:** Hematological changes (X±SE) in calves before and after treatment (n=15).

| Parameters | Before treatment | After treatment | p value |
|------------|------------------|----------------|---------|
| WBC (10^9/L) | 16.30±1.25      | 11.47±1.09     | p<0.01  |
| LYM (10^9/L)  | 6.05±0.83       | 3.5±0.38       | p<0.01  |
| RBC (10^12/L) | 9.07±0.42       | 11.01±0.84     | p>0.05  |
| MCV (fl)    | 44.57±1.13      | 41.25±0.96     | p<0.05  |
| HCT (%)     | 50.64±2.7       | 49.9±10.57     | p>0.05  |

WBC=White blood cell, RBC=Red blood cells, MCV=Mean corpuscular volume, HCT=Hematocrit, LYM=Lymphocyte, SE=Standard error
others in neonatal calves with diarrhea and suspected septicemia [15-17]. Treatment significantly corrected to normal values the hematological parameters that were examined with the exception of RBC. Pre-treatment leukocyte count was high because of the inflammation that occurred in the organism, and that the HCT levels were high due to the dehydration that occurred due to diarrhea.

Hepcidin is controlled by the presence of inflammation in the body, Fe storage, and erythropoietic activity in the bone marrow and plays a primary role in the homeostasis of Fe [4]. The increase in tissue and plasma Fe levels stimulates the synthesis of hepcidin and reduces Fe release and enteric Fe absorption from macrophages and hepatocytes [18]. Increased hepcidin concentrations during inflammation and infection reduce serum Fe levels by decreasing Fe release from macrophages and hepatocytes, and thus Fe required for microorganisms and tumor cells is restricted [19].

Serum hepcidin levels in calves with suspected septicemia were significantly high before treatment when compared to after treatment; also Fe levels were lower before treatment when compared to after treatment in this study. This situation could be related to the interaction between hepcidin and Fe and also gives credence to the role of hepcidin in the hemostasis of Fe during inflammation and infection. As in our study, Fe levels are well known to decrease in diarrheic calves when compared to healthy calves [20,21]. Although no study exists reporting hepcidin concentration in diseased calves, studies in human subjects show that cord blood hepcidin levels might be an important indicator in diagnosing early-onset of neonatal sepsis. The cord blood levels of neonatal infants with sepsis varied between 118.1 and 8400 ng/mL and were significantly higher than the healthy infants [22]. A similar result was reported that hepcidin concentrations in neonatal infants with sepsis were significantly higher than in healthy infants [23]. These findings along with our results add credence to the idea that hepcidin-Fe interaction may play a role in the pathogenesis of septicemia.

The production of free oxygen species causes alterations in protein, lipid, and DNA during oxidative stress and leads to the development of lesions in the organs [24]. Free iron has toxic characteristics as it catalyzes the production of ROSs [25] and thus causes oxidative stress [26]. The role of Fe in the development of oxidative stress may once more show the importance of hepcidin, as an important Fe regulator, with regard to enhancing antioxidant capacity through inhibiting utilization of Fe by the organism as well as the host cells.

The antioxidant and oxidative system are in a constant state of balance in the organism. Any event breaking up this balance in favor of the oxidative stress molecules will cause cell damage [27,28]. The host cells initiate the antioxidant system in case of exposure to oxidative stress [27]. Kabu et al. [16] reported TOS and TAS values in neonatal calves with diarrhea as 13.47±0.81 μmol H₂O₂/L and 0.51±0.02 mmol Trolox-equivalent/L, respectively, and treatment of these calves caused changes in these values of 11.21±0.26 μmol H₂O₂/L and 0.55±0.02 mmol Trolox-equivalent/L, respectively. Studies also reported that parameters used for oxidative stress (malondialdehyde) were higher [29] and antioxidant parameters (superoxide dismutase [21], TAS) were lower in diarrheic calves [29]. Similarly, in our study, TAS level was significantly lower and TOS level was significantly higher in diarrheic calves before treatment, and treatment caused corrections in these parameters. Decrease in TAS and increase in TOS levels demonstrated that oxidative stress was evident in the diseased calves in our study. Increased TOS and hepcidin levels before treatment are thought that associated with inflammation. After treatment increased TAS and decreased hepcidin levels support this opinion.

**Conclusion**

Hepcidin may play an important part in non-specific immunity and is a key molecule that plays a role in the pathogenesis of diseases by enhancing the development of antioxidant system. However, more detailed studies are needed on the role of hepcidin in the pathogenesis of septicemia.

**Authors’ Contributions**

This work was carried out in collaboration between all authors. EEE, HME and AHK: Designed the experimental procedures. EEE, EG and MK: Conducted the research work. EEE, AHK, MO and AK: Helped in laboratory analysis. All authors read and approved the final manuscript.

**Acknowledgments**

This study was financially supported by the Scientific Research Projects Coordinatorship of Kafkas University under the project number 2014-VF-37.

**Competing Interests**

The authors declare that they have no competing interests.

**References**

1. Constable, P.D. (2004) Antimicrobial use in the treatment of calf diarrhea. *J. Vet. Intern. Med.*, 18: 8-17.
2. Citil, M. and Gokce, E. (2013) Neonatal septicemia. *Türk. Klin. J. Vet. Sci.*, 4: 62-70.
3. Park, C.H., Valore, E.V., Waring, A.J. and Ganz, T. (2001) Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J. Biol. Chem.*, 276: 7806-7810.
4. Singh, B., Arora, S., Agrawal, P. and Gupta, S.K. (2011) Hepcidin: A novel peptide hormone regulating iron metabolism. *Clin. Chim. Acta*, 412: 823-830.
5. Ganz, T. and Nemeth, E. (2012) Hepcidin and iron homeostasis. *Biochim. Biophys. Acta*, 1823: 1434-1443.
6. Ganz, T. (2003) Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood*, 102: 783-788.
7. Galaris, D. and Pantopoulos, K. (2008) Oxidative stress and iron homeostasis: Mechanistic and health aspects. *Crit. Rev. Clin. Lab. Sci.*, 45: 1-23.

8. Falzacappa, M.V.V. and Muckenhalter, M.U. (2005) Hepcidin: Iron-hormone and anti-microbial peptide. *Gene*, 364: 37-44.

9. Berger, L.L. (1996) Trace minerals: Keys to immunity. *Salt & Trace Minerals*, 28: 1-4.

10. Albera, E. and Kankofer, M. (2009) Antioxidants in colostrum and milk of sows and cows. *Reprod. Domest. Anim.*, 44: 606-611.

11. Lykkesfeldt, J. and Svendsen, O. (2007) Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Vet. J.*, 173: 502-511.

12. Radostitis, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2006) General systemic states. In: Radotits, O.M., editor. *Veterinary Medicine, Textbook of the Disease of Cattle, Horses, Sheep, Pigs and Goats*. 10th ed. Saunders Elsevier, Philadelphia, PA. p51-52.

13. Fecteau, G., Smith, B.P. and George, L.W. (2009) Septicemia and meningitis in the newborn calf. *Vet. Clin. North. Am. Food Anim. Pract.*, 25: 195-208.

14. Knowles, T.G., Edwards, J.E., Bazeley, K.J., Brown, S.N., Butterworth, A. and Warriss, P.D. (2000) Changes in the blood biochemical and haematological profile of neonatal calves with age. *Vet. Rec.*, 147: 593-598.

15. Ocal, N., Dura, S.Y., Yagci, B.B. and Gazyagci, S. (2006) Field condition diagnosis and treatment of acid-base balance disorders in calves with diarrhea. *Kafkas Univ. Vet. Fak. Derg.*, 12: 175-183.

16. Kabu, M., Cigerci, I.H., Uyarlar, C. and Celik, H.A. (2013) Determination of oxidative status and oxidative DNA damage in calves with diarrhea before and after treatment 10th National Veterinary Internal Medicine Congress, Nevşehir, Turkey; 27-30 June, 2013. p91-92.

17. Basoglu, A., Baspinar, N., Tenori, L., Hu, X. and Yildiz, R. (2014) NMR based metabolomics evaluation in neonatal calves with acute diarrhea and suspected sepsis: A new approach for biomarkers. *Metabolomics*, 4: 1-6.

18. Donovan, A., Lima, C.A., Pinkus, J.L., Pinkus, G.S., Zon, L.I., Robine, S. and Andrews, N.C. (2005) The iron exporter ferroportin/Sle40a1 is essential for iron homeostasis. *Cell Metab.*, 1: 191-200.

19. Vyoral, D. and Petrak, J. (2005) Hepcidin: A direct link between iron metabolism and immunity. *Int. J. Biochem. Cell Biol.*, 37: 1768-1773.

20. Uzlu, E., Karapcihliyan, M., Citil, M., Gokce, E. and Erdogan, H.M. (2010) Investigation of serum sialic acid and some biochemical parameters in calf with diarrhea symptoms *Y. Y. U. Vet. Fak. Derg.*, 21: 83-86.

21. Ghanem, M.M., El-Fkhrayn, S.F., Abd-El Raof, Y.M. and El-Attar, H.M. (2012) Clinical and haematobiochemical evaluation of dirrheic neonatal buffalo calves (*Bubalus bubalis*) with reference to antioxidant changes. *Benha Vet. Med. J.*, 23: 275-288.

22. Cizmeci, M.N., Kara, S., Kanburoglu, M.K., Simavli, S., Duvan, C.I. and Tatli, M.M. (2014) Detection of cord blood hepcidin levels as a biomarker for early-onset neonatal sepsis. *Med. Hypotheses*, 82: 310-312.

23. Wu, T., Tabangin, M., Kusano, R., Ma, Y., Ridsdale, R. and Akinbi, H. (2013) The utility of serum hepcidin as a biomarker for late-onset neonatal sepsis. *J. Pediatr.*, 162: 67-71.

24. Loréal, O., Cavey, T., Bardou-Jacquet, E., Guggenbuhl, P., Kopert, M. and Brissot, P. (2014) Iron, hepcidin, and the metal connection. *Front. Pharmacol.*, 5: 1-10.

25. Ganz, T. (2005) Hepcidin-a regulator of intestinal iron absorption and iron recycling by macrophages. *Best Pract. Res. Clin. Haematol.*, 18: 171-182.

26. Kumar, P. and Ashok, V. (2014) Role of iron in the oxidative stress in the pathophysiology of endometriosis: A new concept to know the potential therapeutic benefit. *Fertil. Sci. Res.*, 1: 19-22.

27. Mercan, U. (2004) Importance of free radicals in toxicology. *Y. Y. U. Vet. Fak. Derg.*, 15: 91-96.

28. Tahakoglu, E. and Durgut, R. (2013) Oxidative stress in veterinary medicine and effects in some important diseases in veterinary medicine and effects in some important diseases. *A. V. K. A. E. Derg.*, 3: 69-75.

29. Yilmaz, S., Isi, M., Kandemir, M.F. and Gul, Y. (2014) Malondialdehyde and total antioxidant levels and hematological parameters of beef cattle with coccidiosis. *Y. Y. U. Vet. Fak. Derg.*, 25: 41-45.