Original Research

Assessment of Role of Hepcidin in Inflammatory Condition in Canine Pyometra

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Abstract

Hepcidin is one of the great discovery in hematology, associated with iron homeostasis. It is a peptide of 25 amino acids and it is synthesized primarily in the liver, which has its synthesis linked to infections and inflammations. Pyometra is a common reproductive disorder in the middle-aged bitches in diestrual phase of estrous cycle when uterus is being exposed to high levels of progesterone. Pyometra is associated with bacterial infection leading to endometritis and toxaemia. Pyometra develops as a result of complex etiological factors which includes hormonal influence of uterine environment, virulence of the infecting bacteria with concomitant disability of the bitches to combat the infection or its inflammatory products. This accelerates the inflammatory process in the affected bitches and activates the macrophages to scavenge the pathogenic bacteria. The present study was conducted to explore the role of hepcidin hormone in mediating the inflammatory process in pyometra affected bitches. Blood samples were collected from ten dogs with open type of pyometra and from ten apparently healthy dogs. Haematological parameters and hepcidin were estimated in pyometra affected bitches and in control animals. Leucocytosis and neutrophilia and their blood hepcidin hormone level were elevated when compared to apparently healthy dogs. This shows that increase in hepcidin hormone is beneficial in mediating the inflammatory process because it increases the intracellular iron content of macrophages which is needed for destruction of engulfed pathogens.

Key words: Canine Pyometra, Hepcidin, Inflammation Neutrophilia, Iron, Lymphocytes, Macrophages

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Introduction

Pyometra either open or closed type in dogs is a life threatening disorder. It is most commonly seen in the middle aged bitches in diestrual phase of estrus cycle when uterus is being exposed to high levels of progesterone (Hardy and Osborne, 1974). Pyometra develops as a result of complex etiological factors

which includes hormonal influence of uterine environment, virulence of the infecting bacteria with concomitant disability of the bitches to combat the infection or its inflammatory products. Progesterone is responsible for the functional closure of the cervix and relaxation of the myometrium. Progesterone induced physiological changes greatly increases the susceptibility of the uterus to infection (Borresen, 1975). In response to changes in sex steroids the endometrium undergoes cyclical remodeling, integrating morphological and functional changes which is controlled by cytokines, interleukins and growth factors (Dekel *et al*., 2010). There is a definite hormonal imbalance existing in the course of pyometra and it will cause inflammatory reactions which is getting intensified with the toxemia due to bacteremia that in turn affects the functions of vital organs like liver, kidney, bone marrow etc. (Plavec *et al*., 2006). Hepcidin (Hepc) is a 25-amino-acid protein synthesized by liver which causes internalisation and degradation of ferroportin molecule resulting in reduced efflux of iron from the iron utilization cells like macrophages, enterocytes and bone marrow cells. Presence of inflammation, increased serum iron level and oxidative stress up-regulates the hepcidin expression in the hepatocytes and increases the intracellular iron content. This will accelerates the destruction of pathogens by Fentons reaction (Rajamanickam *et al*., 2017). Based on this hypothesis the present study was conducted to identify the role of hepcidin in canine pyometra because pyometra also cause inflammation.

Materials and Methods

The present study was carried out in ten clinical cases of pyometra in bitches aged between 2-13 years irrespective of the breed presented for treatment at Small Animal Clinic Outpatient unit in Teaching Veterinary Clinical Complex. A clinical diagnosis of pyometra was made in bitches presented on the basis of medical history, clinical examination and haematological studies and the condition was confirmed with the help of ultra-sonographic studies. Ten apparently healthy dogs of same age group in diestrual stage were selected to serve as control group. Blood samples were collected from all the bitches by either cephalic or saphenous vein puncture. From each bitch, 2ml of blood were collected in EDTA (Ethylene diamine tetra acetic) coated vials and 2 ml in a clot activator vial. Blood smears were also prepared to carry out differential count.

All the 10 bitches on the day of presentation to the clinic were subjected to transabdominal ultrasound scanning (Aquilo® Pro, Esaote Piemedicals) by using 5 to 7.5 MHz sector probe. The uterus was examined to evaluate the integrity of the endometrium, presence of exudates and cystic hyperplasia of the endometrium. The hematological parameters such as haemoglobin (Hb) was estimated by acid hematin method, packed cell volume (PCV) by micro haematocrit method, total erythrocyte count (TEC), total leukocyte count (TLC) and platelet count by Hemocytometer method, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were

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calculated using formulae and differential count for leukocyte by blood smear examination using Giemsa stain as per the method given by Coles (1986). Hepcidin hormone present in the serum was estimated as per the procedure given in the ELISA kit supplied by Sincere Biotech, Beijing101300, China. Data obtained were subjected to student's t-test as per the method explained by Snedecor and Cochran (1994).

Results and Discussion

Ultrasonography revealed enlargement of both uterine horns in the bitches which are clinically suspected for pyometra. Sonographically, the lumen of the horns were filled with homogenous anechoic presented fluid or pus filled pocket like appearance (Fig.1). In six bitches, the endometrium appeared thick and irregular and within the thickened endometrium there were islets of anechoic foci representing dilated cystic glands. Four bitches had mild thickening of the endometrium with very little fluid accumulation.

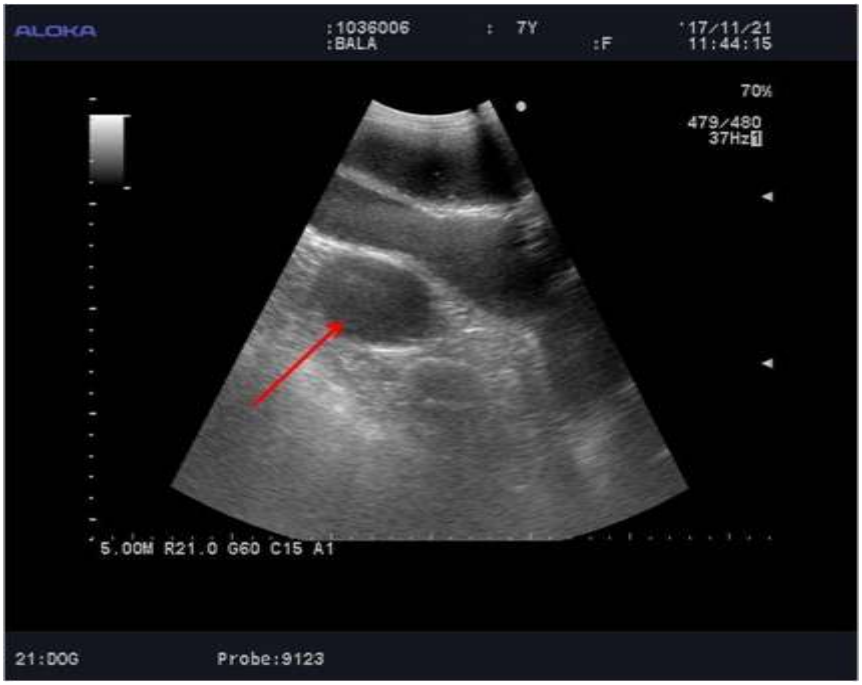

Fig.1: Ultrasonographic picture of canine pyometric uterus (Red arrow indicates the anechoic pocket filled with pus)

The mean and standard error values of haemoglobin concentration, packed cell volume, total erythrocyte count, total leucocyte count, platelets, neutrophils, lymphocytes, monocytes, eosinophils, MCH, MCV, MCHC and hepcidin were summarized in Table 1. Haemoglobin, RBC, platelets, PCV, erythrocyte indices, monocytes and eosinophil's of control and pyometra affected dogs did not differ significantly. Pyometric bitches had leucocytosis (27.08±4.07 $X10^3/mm^3$), neutrophilia (84.50±2.12%) and lymphocytopenia (11.40±1.93 %) when compared to apparently healthy dogs ($P<0.01$). Elevated hepcidin level (51.92±1.08

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ng/ml) was noticed in pyometra affected bitches when compared to apparently healthy dogs (19.62±0.74 ng/ml).

Table 1: Results for haematology and hepcidin hormonal assay in apparently healthy and pyometra affected dogs

Parameters	Apparently Healthy Dogs	Pyometra Affected Dogs
Haemoglobin (g/dl)	11.32 ± 0.54	12.66± 1.00
PCV (%)	36.14 ±1.81	32.49 ± 2.37
RBC($x10^6/mm^3$)	5.5 ± 0.3	5.26 ± 0.3
WBC($x10^3/mm^3$)	12.39 ± 6.74	27.08 ± 4.07**
Platelets($x10^5/mm^3$)	1.20± 0.32	2.08± 0.4
Neutrophils (%)	72.65 ± 0.49	84.5 ± 2.12**
Lymphocytes (%)	19.8 ± 0.91	11.4 ± 1.93**
Monocytes (%)	1.9 ± 0.45	2.9 ± 0.54
Eosinophils (%)	0.5 ± 0.16	0.4 ±0.22
MCH(pg)	21.69 ± 1.69	23.8 ±0.65
MCV(fl)	62.83 ± 1.72	61.44 ± 1.36
MCHC (%)	31.83 ± 1.56	38.89 ± 1.02
Hepcidin (ng/ml)	19.62±0.74	51.92±1.08**

***Significance at the level of P < 0.01*

In the pyometra affected bitch, there was an enlargement of uterus as well as both the uterine horns. The lumen of the horns were seen filled with homogenous contents and was presented as fluid filled pocket. Sixty per cent of the experimental group had irregular and thickened endometrial morphology. The cystic glands in these bitches were dilated and seen to be fluid filled sacs. Forty per cent of the experimental group showed moderate thickening of the endometrial wall with small quantity of accumulated fluid. All haematological parameters recorded are observed to be within the physiological range. But there exists statistical variation between apparently healthy and pyometra affected dogs in their WBC, neutrophils, lymphocyte andhepcidin respectively. The total leukocyte count was higher in plyometric dogs than the control group.Leukocytosis was the most consistent finding among the bitches affected with pyometra which is in agreement with previous report of Shah *et al.* (2017). Leukocytosis depends upon the severity of inflammation. The increased leukocyte count observed in pyometra cases might be due to cellular immune response to local uterine inflammation (Patil*et al.*, 2013).

Marked neutrophilia in the pyometric bitches was recorded in the present study. Neutrophilia may be due to the retention of purulent exudate in the uterus which exerts a chemotactic effect on neutrophils during the process of phagocytosis thereby causing accelerated granulopoiesis (Dabhi *et al.*, 2009). The lymphocytes were significantly lower in plyometric dogs than the apparently healthy dogs indicating lymphocytopenia. The suppression of lymphocyte activity in the bitches with pyometra in the present study would have been induced by endotoxaemia. A wide range of factors has been proposed to be responsible

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for the inhibition. These include immunoglobins, α globulins, lipoproteins etc. (Barta, 1986). Leucocytosis may also be due to absolute increase in neutrophil count as a result of severe suppurative inflammation of the uterus. The suppression of lymphocyte activity and impaired immune response could have been induced by endotoxaemia and bacterial products (Granowitz*et al*., 1993). Hepcidin level was found to be increased in thepyometra affected bitches this may be due to upregualtion of hepcidingene expression by inflammatory mediators. During inflammatory conditions different type of cytokines will be produced, among them IL6 and IL1 were most potent activators of hepcidin gene expression. They modulates the janus kinase (JAK)/signal transducer and activator of transcription-3 (STAT3) signaling pathway and increase the expression of hepcidin gene. This increases the internalisation of ferroportin receptor in the iron utilisation cells and decreases the release of iron from these cells into the circulation. This increase in intracellular iron content of the iron utilising cells like macrophages accelerates the pathogen destruction through Fentons reaction (Rajamanickam *et al*., 2017). Hence, increase in the hepcidin hormone is beneficial in cleansing the pathogenic microbes from the body system of pyometra affected dogs.

Conclusion

The present study indicates that the increase in the hepcidin hormone mediates the inflammatory process in pyomera affected dogs by increasing the intracellular iron content of the iron utilizing cells and accelerates the phagocytosis process. Hence the role of hepcidin in vital in reducing the septicaemia and in prevention of complications in the pyometra affected dogs.

References

1. Barta O. 1986. Immunomodulatory effect of serum on lymphocytes—consequences for the study of immunostimulants in vitro and in vivo. Comparative immunology, microbiology and infectious diseases, 9(2-3): 193-203.
2. Borresen B. 1975. Pyometra in the dog - a pathophysiological investigation I. The pyometra syndrome, a review. *Nordisk Veterinaermedicin*, 27(10): 508-517.
3. Coles EH. 1986. - Veterinary Clinical Pathology. 4thedn. W.B. Saunders, Philadelphia.
4. Dabhi DM, Dhami AJ. Parikh PV, and Patil DB. 2009. Comparative evaluation of haematological parameters in healthy and pyometra affected bitches. *Indian Journal of Animal Reproduction*, 30(1): 70-72.
5. Dekel N, Gnainsky Y, Granot I, and Mor G. 2010. Inflammation and implantation. *American Journal of Reproductive Immunology*, 63(1): 17-21.
6. Granowitz EV, Porat R, Mier JW, Orencole SF, Callahan MV, Cannon JG and Vannier E. 1993. Hematologic and immunomodulatory effects of an interleukin-1 receptor antagonist coinfusion during low-dose endotoxemia in healthy humans. Blood, 82(10): 2985-2990.
7. Hardy RM, and Osborne CA. 1974. Canine Pyometra: Pathophysiology, diagnosis and treatment of uterine and extra uterine lesions. *Journal of the American Animal Hospital Association*, 10(3): 245-268.
8. Patil AR, Swamy M, Chandra A, and Jawre S. 2013. Clinico-haematological and serum biochemical alterations in pyometra affected bitches. *African Journal of Biotechnology*.12 (13).

DOI 10.5455/ijlr.20180704063915

9. Plavec T, Celinsek B, Dolinar K, PecarJ, Nemec A, and Butinar J. 2006. Haemostasis impairment in bitches with pyometra. *Actaveterinaria*, 56(5-6): 529-540.
10. Rajamanickam K, Leela V, LoganathasamyK,Bhaskaran Ravi Latha andBalagangatharathilagar M, 2017. Hepcidin: Discovery to Destiny. *International Journal of Chemical Studies*, 5(6): 1725-1733.
11. Shah SA, Sood NK, Wani BM, Rather MA, Beigh AB, and Amin U, 2017. Haemato-biochemical studies in canine pyometra. Journal of Pharmacognosy and Phytochemistry, 6(4): 14-17.
12. Snedecor GW and Cochran WG. 1994 -Statistsical Methods, 8thedn. Iowa State University Press, USA.

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