C-REACTIVE PROTEIN (CRP) LEVELS IN PRE AND POST-OVARIOHYSTERECTOMISED DOGS AFFECTED WITH PYOMETRA

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Canine pyometra is a life-threatening disease common in countries where spaying of dogs is not routinely performed. The disease is associated with endotoxaemia, sepsis, systemic inflammatory response syndrome with a mortality rate of 3-4 per cent in dogs. Female dogs each affected with open cervix pyometra (n=18) and closed cervix pyometra (n=18) were utilized for the study. CRP levels were estimated on day 0 (before ovariohysterectomy) and day 5, 10 post ovariohysterectomy. The mean CRP concentrations were significantly higher ($P<0.05$) in dogs suffering with open and closed pyometra as compared with that of healthy dioestrus dogs (n=6) on day 0. The mean CRP concentrations in both open and closed pyometra dogs decreased significantly ($P\geq0.05$) from days 0 to day 5 and 10.

Keywords: Canine pyometra, C - reactive protein, Bitch.

Acute Phase Proteins (APP) are the blood proteins primarily synthesized by hepatocytes as part of the acute phase response in any infection. CRP is one of the acute phase proteins that are produced as an inflammatory reaction in man following tissue damage caused by inflammation, infection or injury (Sabel and Hanson, 1974). C-reactive protein is a typical acute phase protein whose behavior has been well studied in many diseases (Maury, 1985). Since the first detection of CRP in dog (Caspi *et al*., 1984) and methods for quantifying its serum concentration have been introduced and further characterization of its behavior has also been attempted (Ndungu *et al*., 1991). Today, CRP remains an APP of primary interest in humans, where it is a major marker of infection, autoimmune disease, trauma, malignancy, myocardial infarction (Pepys and Hirshfield, 2003). Furthermore, CRP has been proposed as a marker for wellness assessment (Kao *et al*., 2006). Assay of APP provides better sensitivity and specificity in finding whether the inflammation is acute or chronic. In comparison neutrophil counts had much lower sensitivity and specificity (30-70%) (Horadagoda *et al*., 1999). In a review of more than 900 cases of inflammation in dogs with various diseases, CRP concentrations were significantly correlated with disease, whereas only slight or no correlation was found with total WBC and band neutrophil counts (Nakamura *et al*., 2008).

Materials and Methods

The present study was carried out in female dogs presented to the Department of Veterinary Gynaecology and Obstetrics, Veterinary College, Hebbal, Bangalore as suspected case of pyometra and those cases referred from Department of Veterinary Medicine. A tentative diagnosis of pyometra was made in female dogs on the basis of medical history, physical examination with particular reference to the presence of purulent/ mucopurulent vaginal discharge. Animals exhibiting abnormal vaginal discharge were considered as suspected case of open pyometra (Fig: 1.) and were further examined by transabdominal ultrasonography for confirmation of pyometra. In animals where the clinical signs especially distended abdomen were suggestive of pyometra but did not exhibit any vaginal discharge were tentatively diagnosed as closed pyometra (Fig: 2.). These animals were further subjected to transabdominal ultrasonography for confirmation of the case as closed pyometra (Fig: 3.). Six clinically normal healthy dogs with the history of estrus minimum a month back, served as control for the study were also subjected to ovariohysterectomy as a means of birth

control. Blood samples were collected in sterile vials (Ac Cuvet, Plain Quantum Biologicals, India) before and after the ovariohysterectomy (Day 0, 5 and 10) without anticoagulant and the sera obtained were preserved at -20º C until estimationof CRP.

Fig: 1. Dog showing pus discharge from vagina: open cervix pyometra

Fig: 2. Dog showing visible distension of abdomen in closed pyometra

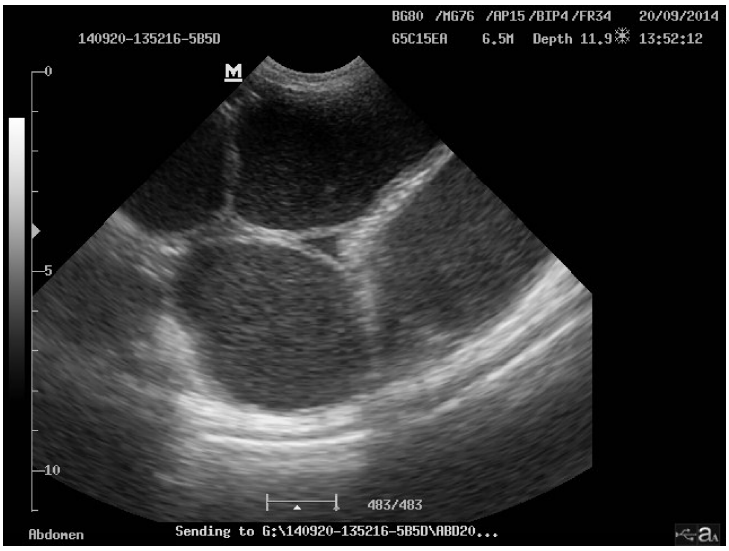

Fig: 3. Ultrasonography picture in pyometra

Estimation of serum C-reactive protein

C-reactiveprotein was estimated with commercially available canine ELISA (CANINE CRP ELISA KIT, Immunology consultant laboratory, Inc.15862 SW, USA), which is a solid phase sandwich immunoassay (Fig: 4. and 5.). Serum samples and CRP standards serially diluted to a range from 3.12 to 200ng/ml were applied to a micro plate with the wells coated with a monoclonal Anti-canine CRP antibody and incubated for 15 minutes at 37^0C before the plate was decanted and washed with diluted buffer. Anti-canine CRP conjugate was added to the wells and the plate incubated for 15 minutes at 37 ^{0}C before the plate was decanted and washed with diluted buffer. Finally, TMB substrate was added and the absorbance of the wells read in a microtiter plate reader at 450 nm using 630 nm as a reference. A standard curve was generated using Microsoft® Excel software and the concentration of the samples was obtained by inserting mean optical density into the function created by the standard curve.

Fig: 4. C-reactive protein kit

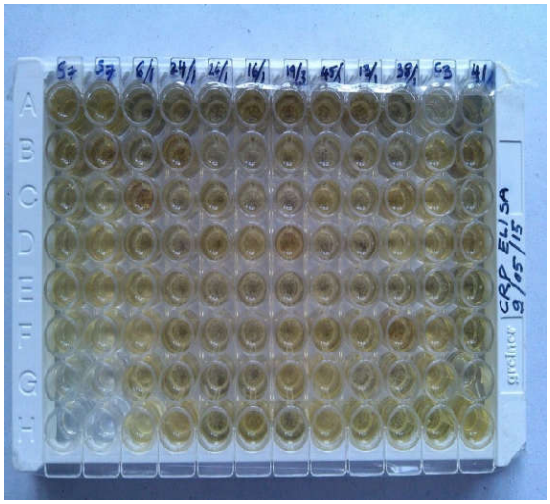

Fig: 5. Estimation of CRP by Elisa

Statistical Analysis

The data generated from the clinical trials was tabulated and the descriptive statistics were computed as per the standard methods. Two way analysis of variance was performed to test the variations between the groups and within the groups using the statistical software SPSS (IBM SPSS 20.0).

Results and discussion

The mean CRP levels of 5.20 ± 0.60, 47.00 ± .80 and 28.00 ± 1.60 µg/ml, respectively were observed in control dioestrus dogs on day 0, day 5 and day 10 post ovariohysterectomy. The mean CRP concentrations recorded on days 5 and 10 were significantly higher following ovariohysterectomy. The CRP levels in dogs with open pyometra were81.00 ± 6.90, 48.00 ± 3.50 and 30.00 ± 2.30 µg/ml, respectively. While corresponding values of 127.00 ± 11.00, 65.00± 4.40 and 39.00 ± 2.00 µg/ml were noticed in dogs with closed pyometra on day 0, 5 and 10, respectively (Table- 1). The mean CRP concentrations were significantly higher ($P<0.05$) in dogs suffering with open and closed pyometra as compared with that of healthy dioestrus dogs on day 0. Further, the mean CRP concentrations increased significantly ($P\leq0.05$) following ovario-hysterectomy in normal healthy dioestrus dogs as compared to their respectively concentrations before surgical intervention. However, the mean CRP concentrations in both open and closed pyometra dogs decreased significantly ($P\geq0.05$) from days 0 to day 5 and 10 as compared to its level on day 0 (before ovariohysterectomy).

Table- 1: Mean CRP (µg/ml) in control and pyometra affected dogs during pre and post ovario-hysterectomy

DAYS	Control (N=6)	Open pyometra (N=18)	Closed pyometra(N=18)
0	5.20 ± 0.60^{xa}	81.00 ± 6.90^{xb}	127.00 ± 11.00^{xc}
5	47.00 ± 2.80^{ya}	48.00 ± 3.50^{yab}	65.00 ± 4.40^{yb}
10	28.00 ± 1.60^{xya}	30.00 ± 2.30^{za}	39.00 ± 2.00^{za}

Note: Common superscript in row abc; Common superscript in column xyz
Means bearing common superscript either in row or column do not differ significantly ($P<0.05$)

Enginler *et al*. (2014); Jitpean *et al*. (2014) have been previously reported the elevated concentrations of CRP in bitches with pyometra in blood samples prior to the surgery and thus proposed to be a useful marker in pyometra and/or other diseases leading to systemic inflammation. The elevated level of CRP in bitches with pyometra before surgery occurs as result of chronic inflammation which as also reported

by Dabrowski *et al*. (2007). Thus, the results of the present study confirm earlier reports of Franssone*t al*. (2007); Dabrowski *et al*. (2007) also.

On the other hand, significant increases in mean CRP levels were observed in dioestrus dogs on day 5 and 10 as compared to Day 0 in the present study. This might have resulted from tissue discontinuity reflecting the severity of inflammatory processes as also mentioned by Serin and Ulutas (2010). Further, Ranganath and Senthil (2007) reported that C-reactive protein levels peaked at 24 hours following ovariohysterectomy in mongrel bitches. In the present study, the serum CRP concentrations were found significantly higher in bitches with pyometra which was in accordance with the reports of Fransson *et al.* (2007). In the present study, higher CRP concentrations were associated with closed pyometra dogs. Pyometra often induces sepsis, depression, anorexia, polydipsia and polyuria which are common clinical signs in bitches with pyometra, emphasizing the systemic effects of the illness in the studied bitches.

Conclusions

The results of the present study indicated that mean acute-phase protein concentrations differ according to pyometra type/severity and suggesting the possible use of peripheral blood levels of CRP to monitor inflammation during the course of the disease.

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