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Research Article

PROGNOSTIC MARKERS OF CANINE PYOMETRA

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ABSTRACT

The objective of the present study is to identify the markers associated with mortality in dogs affected with pyometra. A total of 75 dogs with pyometra were included in the study and were treated surgically. The dogs were retrospectively categorized into 2 groups: Group I (non- survivors) and Group II (survivors). The physiological, haematological and serological findings were compared between the groups. The association of variables such as type of pyometra, systemic inflammatory response syndrome (SIRS), and laboratory parameters with survivability were analysed. Among the variables studied SIRS (P<0.0072), closed pyometra (P<0.0279), elevated alkaline phosphatase (ALP) (P<0.0109), creatinine (P<0.0002) and elevated Serum Amyloid A (SAA) (P<0.0001) were associated with survivability and could be used as prognostic indicators of canine pyometra.

Keywords: Canine, CRP, Prognostic markers, Pyometra, SAA

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Pyometra is one of the most common reproductive diseases in adult intact sexually matured she dogs (Kempisty *et al.*, 2013). It is characterized by accumulation of purulent material within the uterus, leading to a variety of clinical symptoms, and is life-threatening in severe cases (Johnston *et al.*, 2001). Though the etiology of pyometra is complex and still not fully understood, both hormonal and infectious factors might contribute to its development (Dabrowski *et al.*, 2013). *Escherichia coli* is the most common bacterium isolated from the pyometra-affected uterus (Singh *et al.*, 2019). The release of lipopolysaccharide (LPS) from the cell wall of *E. coli* leads to release of proinflammatory cytokines and other chemical mediators, which successively trigger hepatic production of positive acute phase proteins (APPs) (Dabrowski *et al.*, 2009; Jitpean *et al.*, 2014a). Blood concentrations of APPs have been widely used in human medicine as a diagnostic and prognostic tool for sepsis (Wang *et al.*, 2012). The two major APPs in dogs are C-reactive protein (CRP) and serum amyloid A (SAA). Among the two APPs, CRP is assessed most often in canines (Gebhardt *et al.*, 2009; Karlsson *et al.*, 2012) while SAA is not yet widely used. However, few studies reported that the blood concentrations of SAA were elevated in pyometra (Dabrowski *et al.*, 2009; Jitpean *et al.*, 2014a). Limited studies have evaluated the role of different markers in the prognosis as well as propose new prognostic markers of canine pyometra (Jitpean *et al.*, 2014a; Sant'Anna *et al.*, 2014). The aim of this study was to evaluate variables such as hematological and biochemical changes and the role of acute phase proteins CRP and SAA as possible prognostic markers in canine pyometra.

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MATERIALS AND METHODS

The present study involved 75 she dogs aged between 2 to 15 years with the presumptive diagnosis of pyometra presented to the Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram. All the dogs were subjected to a detailed clinical examination and the data regarding the breed, age, parity and clinical signs were collected. The preliminary diagnosis of pyometra was made based on case history, findings of physical examination and diagnostic imaging by either abdominal ultrasonography or radiography or both. She dogs were divided into two groups retrospectively: Group I (non-survivors), Group II (survivors). Except for 9 dogs whose clinical condition was worse and unfit for surgery, other 66 dogs were subjected for ovariohysterectomy (OHE). Before surgery the dogs were treated with inj.Taxim @ 30mg/kg b.wt, I/V, inj.Vomikind @0.2 mg/kg I/V and fluid therapy. Post operatively treated with inj.Taxim @ 30 mg/kg b.wt, I/V upto 5 days, Tab. Pantoprozole @ 1 mg/kg b.wt. PO, Inj.Melonex @ 0.2 mg/kg b.wt for 3 days and fluid therapy for 3 days. The diagnosis was confirmed by postoperative macroscopic examination of uterus and histopathological examination of uterine tissues.

Collection of samples: Blood samples were collected aseptically using sterile EDTA vacutainers from cephalic and/or saphenous vein. Further 5 ml of blood was collected in a sterile vial with clot activator. The serum was separated by centrifugation at 3000 rpm for 7 min. The blood samples were processed within 2 hours of collection for the haematological parameters. An aliquot of serum was utilized for estimation of biochemical parameters while the remaining portion was preserved at -20º C for

estimation of biomarkers.

After ovariohysterectomy, the uterus was subjected for macroscopic evaluation and the representative tissue samples of the uterus were collected for histopathology and stored in 10 per cent neutral buffered formal saline until further processing.

Laboratory tests: Hematological (Differential leucocyte count, total leucocyte count, haemoglobin, total erythrocyte count) and biochemical tests (Alanine aminotransferase, Aspartate aminotransferase, total protein, albumin, globulin, A:G ratio, Blood urea nitrogen, creatinine) were performed by adopting standard procedures. C-reactive protein estimation was done by using a latex agglutination kit, used for humans, as per the manufacturer's recommendations (C-Reactive Protein, BioSystems, Barcelona, Spain); Canine Serum amyloid A, GENLISA™, Krishgen Biosystems, Mumbai, India). Sandwich ELISA commercial kit was used for quantitative determination of Canine Serum Amyloid A in serum.

Statistical analysis: The data generated in the study was tabulated and was subjected to Statistical analysis by using SPSS (Software Packages for Social Sciences) 20.00 version as per the methods described by Snedecor and Cochran (1994). The mean values of physiological, hemato-biochemical and serum biomarker values were compared by students t test between Group I and Group II. For the association of variables SIRS, type of pyometra and laboratory parameters with survivability Fisher exact test was used.

RESULTS AND DISCUSSION

Out of 75 dogs enrolled in the present study, 14 dogs succumbed to death accounting to a mortality rate of 18.67 per cent. Among the 14 dogs, only 5 dogs (6.67%) died after surgery, while 9 dogs (12.00%) died during the course of the disease. The post mortem diagnosis for cause of death could not be made as none of the owners were agreed for post mortem. Kuplulu *et al.* (2009) reported a mortality rate of 26.6 per cent within 21 days after surgery in pyometra affected she dogs. Mortality post-surgery might be higher when the disease was diagnosed at later stages during the course of the disease.

The mean values of clinical and laboratory parameters of non-survivors and survivors in pyometra affected she dogs are presented in Table 1. The association of various variables with mortality was analysed and their frequencies are presented in Table 2. The variables considered for interpretation of association included SIRS, type of pyometra and laboratory parameters that showed a significant difference between survivors and non-survivors by independent sample 't' test. Out of 75 she dogs with pyometra 70.67 percent (n=53) of the she dogs were positive for SIRS.

In the present study, a significant association with mortality was noticed with the presence of SIRS ($P<0.0072$), closed pyometra ($P<0.0279$), elevated ALP ($P<0.0109$), elevated creatinine ($P<0.0002$) and elevated serum amyloid A ($P<0.0001$) in the pyometra affected she dogs, which was confirmed on the basis of Fisher exact test (Table 2). The association of SIRS ($P<0.0072$) with mortality in the present study showed the importance of this criterion so that supportive treatment could be instituted at the earliest. Previous studies reported that there was a significant association between the presence of SIRS in pyometra and creatinine levels above 2.5 mg/dl with increased morbidity and mortality in pyometra (Fransson *et al.*, 2007; Sant'Anna *et al.*, 2014).

In the present study, association of closed pyometra ($P<0.0279$) with mortality revealed that closed pyometra was likely to result in poor prognosis. The possible explanation for the present finding was that SIRS was more commonly associated in dogs with closed cervix pyometra compared to open cervix pyometra. In the present study 80.00 per cent of she dogs with closed cervix pyometra had positive SIRS status. Another reason for poor prognosis in closed pyometra she dogs might be attributed to delay in presenting the case for veterinary aid. On the contrary, Sant'Anna *et al.*, (2014) and Jitpean *et al.* (2017) reported no difference in the prognosis for dogs with open or closed pyometra.

The mean values of hematological parameters like Hb, TEC were significantly higher ($P<0.05$) in survivors while percentage of band neutrophils were significantly higher ($P<0.05$) in non-survivors. The mild to moderate normocytic, normochromic anaemia recorded in pyometra represented chronic inflammation and toxic effects of bacterial agents on the bone marrow (De Schepper *et al.*, 1987). Feldman (2000) reported that absolute neutrophilia with shift to left was common due to infection and septicaemia in canine pyometra. However, the association of these variables with mortality was not significant in the present study. These results showed that though altered hematological parameters are useful in diagnosis, they might not have prognostic importance in canine pyometra.

Out of 14 she dogs that succumbed to death in the present study, leucopenia was noticed in 2 she dogs (2/14). Increased mortality has also been demonstrated in animals with leucopenia which might be to be increased susceptibility to sepsis and secondary diseases due to impaired immune system (Jitpean *et al.*, 2014c). The mean values of biochemical parameters *viz*., ALP ($P<0.01$),

BUN (P<0.01), creatinine (P<0.01), total protein (P<0.05), globulin (P<0.05) were significantly elevated in non-survived she dogs than survived she dogs with pyometra. However, among these parameters significant association with mortality was observed with alkaline phosphatase (P<0.0109) and creatinine (P<0.0002). Similar to the present study the elevated BUN and creatinine were recorded by Kuplulu *et al.* (2009), Sant'Anna *et al.* (2014) and Demirel *et al.* (2018) in pyometra affected she dogs which required prolonged hospitalization and/or mortality. Kuplulu *et al.* (2009) opined that elderly azotemic dogs with BUN concentration above 30 mg/dl accompanied with over 10.0 per cent band neutrophils would likely end up with mortality due to cardiovascular complications within 3 days after surgery. In conflict to these reports, elevated concentration of serum alkaline phosphatase activity was recorded in the present study which had a significant association with mortality. The elevated serum alkaline phosphatase probably reflected intrahepatic cholestasis (Sevelius *et al.*, 1990). Elevated creatinine in the present investigation was strongly associated with mortality in canine pyometra (P<0.0002). Serum creatinine would be elevated when approximately 75.0 per cent of nephrons become non-functional (Chen *et al.*, 2017). Therefore, the ability to cope with illness and treatment was impaired in dogs with elevated blood creatinine concentrations. Sharma (2004) observed poor prognosis in pyometra she dogs that recorded blood urea nitrogen concentration greater than 120 mg/dl and creatinine greater than 5 mg/dl. Based on the results from the present study and other investigations, preoperative estimation of serum creatinine was valuable to forecast the outcome especially in older and systemically affected she dogs. The mean total protein and globulin were significantly higher in non-survived than survived she dogs. Hagman (2004) reported that α2-, β1- and γ-globulin fractions of serum proteins in the pyometra she dogs were significantly increased when compared with the controls might be due to increased synthesis of acute phase proteins and antibodies in response to bacterial infection.

In the present study, the CRP and SAA concentrations were increased in both non-survivors and survivors. However, the mean CRP concentration did not vary between non-survivors and survivors. Earlier studies reported that elevated CRP was associated with SIRS and prolonged hospitalization in she dogs with pyometra. Fransson *et al.* (2007) observed that a plasma CRP concentration of >276.35 mg/L was associated with hospitalization exceeding 2 days. In contrast, Jitpean *et al.* (2014a) reported no significant difference in she dogs with

Table 1

Clinical and laboratory parameters of non-survivors and survivors in pyometra affected she dogs (Mean±SE)

S. No.	Parameter		Non survivors (n=14)	Survivors (n=61)
1	Temperature (ºF)		102.92±0.39*	102.29±0.10
2	Heart rate (bpm)		118.57±4.22*	107.00±1.39
3	Respiratory rate(breaths/ min)		32.50±3.78	24.52±0.90
4	Hemoglobin (g/dl)		9.06±0.66	10.28±0.25*
5	PCV (%)		29.43±2.34	31.99±0.85
6	TEC ($x10^6$/µl)		4.87±0.33	5.53±0.13*
7	TLC ($x10^3$/µl)		21.96±3.48	20.64±1.16
8	DLC	Neutrophils (%)	86.93±2.28	86.61±0.70
		Band neutrophils (%)	28.14±1.84**	17.12±1.10
		Lymphocytes (%)	10.86±2.01	10.78±0.56
		Monocytes (%)	1.43±0.34	1.66±0.19
		Eosinophils (%)	0.86±0.23	1.08±0.17
14	Total protein (mg/dl)		8.68± 0.51*	7.25±0.16
15	Albumin (mg/dl)		2.94±0.15	2.65±0.07
16	Globulin (mg/dl)		5.74±0.47*	4.61±0.18
17	A/G ratio		0.55±0.05	0.64±0.04
9	ALT (U/L)		43.02±5.18	35.62±2.29
10	AST (U/L)		55.48±6.34	45.79±2.55
11	ALP (U/L)		206.45±28.08**	123.03±8.83
12	BUN (mg/dl)		114.61±16.84**	33.08±2.82
13	Creatinine (mg/dl)		5.71±0.88**	1.65±0.10
18	CRP (mg/L)		87.43±13.69	67.38±6.90
19	SAA (mg/L)		88.93±1.74**	54.19±1.98

Significant association (*P<0.05; **P<0.01)

Table 2

Association of SIRS, type of pyometra and laboratory parameters with survivability by using Fisher exact test

S. No.	Variables		Non-survivors/Total no. of animals affected	P value
1	SIRS	Presence	14/53 (26.41%)	0.0072*
		Absence	0/22	
2	Closed pyometra	Presence	6/15 (40%)	0.0279*
		Absence	8/60 (13.33%)	
3	Elevated band neutrophils	Presence	14/68 (20.58%)	0.3356
		Absence	0/7	
4	Altered haemoglobin	Presence	13/63 (20.63%)	0.4442
		Absence	1/12 (8.33%)	
5	Reduced TEC	Presence	13/57 (22.80%)	0.1653
		Absence	1/18 (5%)	
6	Elevated ALP	Presence	9/25 (36%)	0.0109*
		Absence	5/50 (10%)	
7	Elevated creatinine	Presence	14/43 (32.55%)	0.0002*
		Absence	0/32	
8	Elevated BUN	Presence	13/54 (24.07%)	0.0953
		Absence	1/21 (4.76%)	
9	Altered total protein	Presence	11/43 (25.58%)	0.1323
		Absence	3/32 (9.37%)	
10	Altered globulin	Presence	11/45 (24.44%)	0.1407
		Absence	3/30 (9.99%)	
11	Elevated serum amyloid A	Presence	14/40 (35.0%)	<0.0001*
		Absence	0/35	

*Significant association

prolonged post-operative hospitalization from those with normal post-operative hospitalization. The mean SAA concentration was significantly (P<0.01) higher in non-survivorsthan survivors. Further, SAA had a significant association (P<0.0001) with mortality in pyometra she dogs. The findings of the present study were in accordance with previous investigation of Jitpean *et al*. (2014b) who also reported that SAA was clinically superior in terms of diagnostic or prognostic value compared to CRP in she dogs affected with pyometra. Despite both CRP and SAA being major APPs in canines both have different diagnostic abilities and is in agreement with the results of present study.

The study reports the usefulness of various parameters as prognostic indicators in canine pyometra. Closed pyometra, presence of SIRS, elevated creatinine, ALP and SAA had significant association with mortality and suggest the possibility of using these markers as prognostic indicators of canine pyometra.

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