



Effect of systemic inflammatory response syndrome (SIRS) on prostaglandin metabolite and oxidative stress in canine pyometra

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ABSTRACT

Pyometra, either open or closed cervix, inevitably progresses to systemic inflammatory response syndrome (SIRS), if ovariectomy is not done timely. The aim of the study was to investigate the effect of pyometra led SIRS on certain serum biochemical and prostaglandin metabolite, 13,14-dihydro-15-keto PGF₂ alpha (PGFM) and oxidative stress indices in the canine. The pyometra positive bitches were categorized as SIRS+ (n=29) and SIRS- (n=16) based on temperature (<100.5 or >102.5°F), respiration rate (>20/min), heart rate (>102/min), and total leukocytes count (<6×10³ or >16×10³ per μL). The SIRS+ bitches showed hypoalbuminemia, hyperglobulinemia, elevated blood urea nitrogen and creatinine, decreased super oxide dismutase (SOD) activity with moderate increase in the lipid peroxidation. Further, the SIRS+ bitches had significantly higher serum PGFM concentration (6.83±0.7 vs. 4.12±0.4 ng/mL) than SIRS- and the level was influenced by cervical patency. It was concluded that elevated serum PGFM along with hyperglobulinemia, blood urea nitrogen, creatinine would be useful in diagnosis and monitoring of pyometra led SIRS in bitch.

Keywords: Dog, PGFM, Pyometra, SIRS

Cystic endometrial hyperplasia (CEH) - pyometra complex is a hormonally mediated diestrus disorder of the intact bitch. The prolonged progesterone stimulation of the uterus in the absence of pregnancy results in the endometrial proliferation and secretion, myometrial quiescence and suppression of innate uterine defense resulting in bacterial growth (Sugiura *et al.* 2004). The effects are cumulative, with each infertile estrous cycle exacerbating the uterine disease. Nulliparous bitches of >4 years of age are at high risk of developing pyometra (Fransson *et al.* 2007) and are diagnosed at 7.25–9.36 years of age (Johnston *et al.* 2001). The typical clinical presentation of pyometra includes inappetence, vomiting, polydipsia, lethargy and abdominal distension along with leukocytosis with left shift, normocytic normochromic non-regenerative anaemia and azotemia (Johnston *et al.* 2001). Distension and sacculations of the uterus and endometrial thickening with or without cystic changes in the ultrasonography confirm the diagnosis (Bigliardi *et al.* 2004).

Clinically, pyometra is either open or closed-cervix based on the presence or absence of purulent vaginal discharge. In pyometra, the systemic release of bacterial endotoxin triggers the overproduction of inflammatory mediators and acute phase proteins (Dabrowski *et al.* 2007, Hagman *et al.* 2009, Karlsson *et al.* 2012). Among the inflammatory

mediators, elevated concentration of PGFM is considered to have diagnostic value in differentiating pyometra from other uterine diseases (Hagman *et al.* 2006a, Enginler *et al.* 2014). The unabating endometritis induces excess production of reactive oxygen species that can result in oxidative stress. Undiagnosed and untreated pyometra culminate in SIRS, where the local inflammatory reaction induces the release of endogenous inflammatory mediators that compromise homeostatic mechanism (Hagman 2016). It is reported that pyometra associated with SIRS increased days of hospitalization with lower survival, which is due to multi-organ dysfunction syndrome (Jitpean 2015). The present study aimed to evaluate the effect of SIRS on serum PGFM concentration and oxidative stress markers in canine pyometra.

MATERIALS AND METHODS

The study was conducted during 2017–18 following standard operating guidelines of the Institute Animal Ethics Committee at Veterinary Gynaecology and Obstetrics unit, Referral Veterinary Polyclinic, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India.

Case history: A total of 45 clinical cases of intact bitches with signs of pyometra which includes lethargy, anorexia, polydipsia, polyuria, vomiting with copious muco-purulent vaginal discharge or abdominal distention for a period of 3 to 10 days without any vaginal discharge were included in

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the study. The parity, age and breed were recorded. Transabdominal ultrasonography was done using a curvilinear probe of 3–5 MHz frequency to record the uterine changes for confirmatory diagnosis. Distension of the uterus with accumulation of anechoic fluid with small echogenic particles, thickening of endometrium with or without cystic changes were consistent in pyometra cases.

Categorization: Based on rectal temperature (<100.5 or >102.5°F), respiration rate (>20/min), heart rate (102/min), total leukocytes count (<6×10³ or >16×10³ per μL), the pyometra positive bitches were categorized into SIRS+ (n=29) and SIRS- (n=16) as described by Hauptman *et al.* (1997). Further, based on the presence or absence of purulent discharge in per vaginal examination, the positive cases were categorized into open (n=30) and closed (n=15) cervix pyometra. Following confirmatory diagnosis, blood sample of about 5 mL was collected after taking consent from the owner and serum was separated and stored at –20°C until assay.

Serum biochemical assay: Total protein, albumin, bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen and creatinine concentration in serum were estimated using commercial kits validated for canine. Serum lipid peroxidation (LPO) was determined to estimate the oxidative stress through malondialdehyde production assay (Suleiman *et al.* 1996), total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain 1999) and the activity of superoxide dismutase (SOD) as reported earlier (Madesh and Balasubramanian 1998). Estimation of serum PGFM was done by competitive ELISA using commercial canine specific kit (Bluegene, China).

Statistical analysis: The effect of SIRS on the serum biochemical variables and oxidative stress parameters was analyzed by independent t-test. The effect of SIRS and cervical patency on serum PGFM was analyzed by two-way Anova with Bonferroni test to compare the pair-wise difference. The association between SIRS and cervical patency was determined by Fisher's exact test. Significance was set at 95% level and Graphpad Prism 5.0 was used for construction of graphs.

RESULTS AND DISCUSSION

Based on the hospital occurrence, a point prevalence of pyometra in the nulliparous bitch was about 64.4%. The point prevalence of pyometra was 28.9 and 22.2% in Labrador and Spitz, respectively and the susceptibility was high at 7–10 years of age (Fig. 1A-B). The hospital occurrence of pyometra in nulliparous bitches was in conformity with earlier report (Dhaliwal *et al.* 1998). An increased vulnerability of nulliparous and older bitches to pyometra is imputed to repetitive exposure of the uterus to progesterone due to the long luteal phase of the estrous cycle (De Bosschere *et al.* 2002). Pregnancy, however, has a suppressive effect on the development of pyometra (Hagman *et al.* 2011). As infertile estrous cycles result in pseudo-pregnancy and accumulation of uterine fluid, the

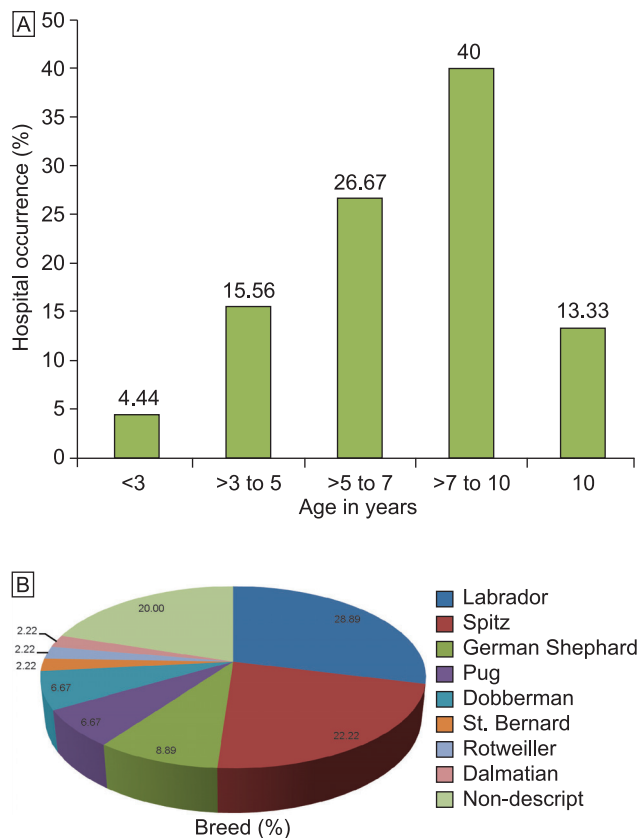


Fig. 1. Hospital occurrence of pyometra with respect to age (A) and breed (B) of the bitch recorded at Referral Veterinary Polyclinic, IVRI, Izatnagar.

parity of the aged bitches needs to be considered. In the present study, the precise information on the parity could not be obtained. An occurrence of 4.4% pyometra in bitches of <3 years could not be explained convincingly as the history did not reveal the use of exogenous steroids. It is reported that administration of high doses of estrogen to terminate pregnancy increases the risk of pyometra in young dogs (Acke *et al.* 2003). With respect to breed, Labrador and Spitz had a higher hospital occurrence of pyometra although breed predisposition is confounded by factors like breed availability in a geographical location and preference of owners for a breed. Multivariate analysis predicted that Rough Collies, Rottweiler, Cavalier King Charles Spaniel, Golden Retriever, Bernese Mountain Dogs and English Cocker Spaniel were at high risk as compared to German Shepherd, Dachshund, and Swedish Hound (Egenvall *et al.* 2001).

Analysis of clinical parameters indicated that pyrexia, tachypnea and tachycardia were evident in SIRS+ pyometra ($P<0.05$; Table 1). The increase in temperature, heart rate and respiration in SIRS+ pyometra is due to the systemic presence of endotoxins of *E. coli* cell wall (Krekeler *et al.* 2012) from the infected uterus (Fransson *et al.* 2007). Hypoalbuminemia, hyperglobulinemia, decreased albumin to globulin ratio and azotemia were significant findings in the SIRS+ pyometra. Blood urea nitrogen was five-fold

Table 1. Vital clinical parameters and leukogram in SIRS+ and SIRS- bitches with pyometra

Variable	SIRS		P value
	Positive (n=29)	Negative (n=16)	
Temperature (°F)	102.95±0.35 ^a	101.47±0.41	0.02
Respiration rate (breaths/min)	45.0±7.62 ^b	19.0±1.5	0.001
Heart rate (beats/min)	121.0±5.20 ^b	98.0±2.14	0.001
Total leukocyte count (×10 ³ /μL)	28.33±3.88 ^a	15.07±2.22	0.03
Neutrophil (%)	74.66±3.75	70.44±4.39	0.47
Lymphocyte (%)	21.12±3.72	26.89±4.50	0.33
Monocyte (%)	2.25±0.33 ^a	1.33±0.29	0.05
Eosinophil (%)	1.35±0.50	0.89±0.26	0.44
Basophil (%)	0.62±0.09	0.44±0.04	0.40
Total erythrocytes count (×10 ⁶ /μL)	3.92±0.21	4.17±0.14	0.34
Haemoglobin (g/dL)	10.12±0.86	11.44±0.69	0.29
Packed cell volume (%)	31.11±2.53	35.33±2.14	0.26

Values with * indicate significant difference at ^aP<0.05; ^bP<0.01.

higher in SIRS+ cases. Total protein was within physiological limits; but, was higher in SIRS+ pyometra (P<0.05). Serum ALT and AST were comparable between the groups; however, hyper-bilirubinemia was observed in both the groups (Table 2).

In this study, the serum biochemical parameters showed a clear pattern that is consistent with previous reports on canine pyometra (Kaymaz *et al.* 1999); but, the response was more pronounced in SIRS+ bitches. For instance, marked hypo-albuminemia and increased creatinine suggest not only pre-renal azotemia due to fever, vomiting and inappetence but also renal impairment due to deposition of antigen-antibody complex in the glomerulus (Hagman 2016). This is supported by very high concentration of blood urea nitrogen in the SIRS+ group. It is reported that persistent antigenic stimuli from the infected uterus results in tubulointerstitial inflammation or immune-complex associated glomerulonephritis leading to glomerular and tubular dysfunction (Maddens *et al.* 2011). Hypoalbuminemia and azotemia are usually imputed to dehydration due to fever and vomiting. A decrease in albumin concentration may occur during the later stages of hepatic damage following hyperproteinemia (Melih *et al.* 2012). On the other hand, the SIRS- bitches showed blood urea nitrogen at normal physiological range and creatinine towards the higher side of physiological range indicating minimum renal involvement. Increased total bilirubin in SIRS+ pyometra is consistent with earlier report (Sato *et al.* 2002). The pyometra led endotoxaemia causes intrahepatic cholestasis and alteration of hepatocellular function (Hagman *et al.* 2006b). The concentration of ALT was decreased in both SIRS+ and SIRS- groups as reported by Kaymaz *et al.* (1999); however, in another study, ALT

Table 2. Effect of pyometra led SIRS on serum biochemical and oxidative stress indicators (mean±SEM) in the bitches

Variable	SIRS		P value
	Positive (n=29)	Negative (n=16)	
Total protein (g/dL)	6.6±0.48 ^a	5.0±0.37 ^a	0.02
Albumin (g/dL)	1.80±0.13	2.30±0.16 ^a	0.03
Globulin (g/dL)	4.80±0.44 ^b	2.70±0.30	0.001
Albumin: globulin ratio (A/G)	0.42±0.06	1.20±0.38 ^a	0.03
Total bilirubin (mg/dL)	3.11±0.47	3.92±0.39	0.22
Alanine amino transferase (ALT, U/L)	14.30±4.04	10.77±0.50	0.42
Aspartate amino transferase (AST, U/L)	19.71±3.17	20.95±1.06	0.74
Blood urea nitrogen (mg/dL)	106.28±17.01 ^b	20.48±1.88	0.001
Creatinine (mg/dL)	2.11±0.30 ^b	0.91±0.07	0.001
Lipid peroxidation, LPO (mM)	7.28±1.90	4.36±0.45	0.17
Total antioxidant capacity, TAC (mM)	494.4±73.18	548.29±41.05	0.57
SOD (U/mL)	270.03±21.82	396.34±30.95 ^b	0.01
PGFM (ng/mL)	6.83±0.70 ^b	4.12±0.44	0.003

Values with * indicate significant difference at ^aP<0.05; ^bP<0.01.

was not modulated in bitches with pyometra (Melih *et al.* 2012, Hagman *et al.* 2009).

Oxidative metabolism is increased at the site of inflammation and increased production of oxygen and nitrogen derived free radicals are common in many chronic diseases. Intracellular killing of antigen by the phagocyte is a major source of reactive oxygen species. Oxidative damage was assessed through lipid peroxidation by measuring MDA, which was nearly 1.7 times higher in SIRS+ than SIRS-pyometra cases (Table 2). Ovariohysterectomy is shown to increase the production of thiobarbituric acid reactive substances, bityrosine and formyl-kynurenine in the bitch by 30 days after ovariohysterectomy (Szczubial *et al.* 2015). In addition, the activity of antioxidant enzyme, SOD was significantly low in SIRS+ cases. It is reported that ovariohysterectomy significantly decreased the activity of SOD and glutathione peroxidase in the erythrocytes (Szczubial *et al.* 2015). Surprisingly, total antioxidant capacity did not differ significantly between SIRS+ and SIRS- pyometra cases. In present study, 33.3% bitches had closed pyometra of which 80% were SIRS+. The relation between cervical patency and SIRS status however, was non-significant (P=0.188). Lack of statistical association does not negate the fact that SIRS is sequelae of pyometra in the bitch (Fransson *et al.* 2007). Pyometra with closed cervix accumulate the uterine contents including endotoxin, which get absorbed into the circulation and aggravate the disease.

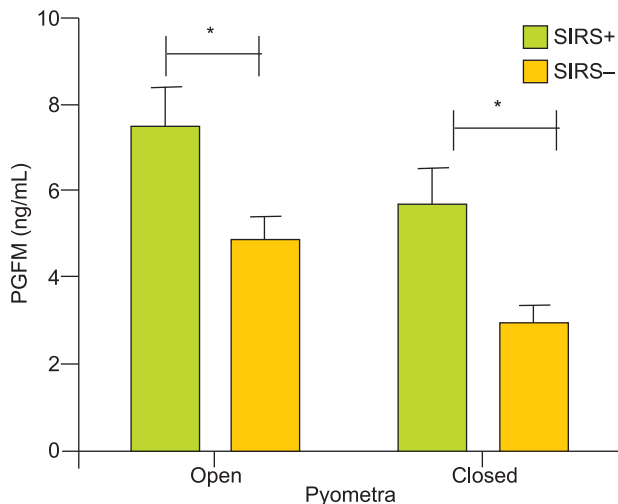


Fig. 2. Effect of cervical patency and SIRS on serum PGFM in the bitches with pyometra. Two-way Anova with Bonferroni *post-hoc* test was done. A significant effect of SIRS status ($P=0.003$) and cervical patency ($P=0.032$) was observed for PGFM.

Patency of the cervix is regulated by circulating estrogen and progesterone and the differential expression of their receptors in the uterus (De Bosschere *et al.* 2003). Monitoring serum pro-inflammatory cytokines, acute phase proteins, endotoxin and inflammatory mediators have the potential in the diagnosis and prognosis of canine pyometra (Dabrowski *et al.* 2007, Hagman *et al.* 2009, Karlsson *et al.* 2012).

Serum PGFM concentration of the bitches with pyometra was influenced by SIRS status ($P=0.003$) and cervical patency ($P=0.032$). The concentration of PGFM was significantly higher by 1.5 to 1.9 times in SIRS+ with open and closed pyometra than SIRS- cases ($P<0.05$; Fig. 2). An increased concentration of PGFM in open-cervix pyometra as compared to closed one could not be explained convincingly and might be due to sustained production of $PGF_{2\alpha}$ from the former. However, a high concentration of PGFM ($e>4,524$ pmol/L) had been reported to differentiate CEH from pyometra (Hagman *et al.* 2006a). Endotoxins released by Gram-negative bacteria stimulate the production of PG and proinflammatory cytokines (Dabrowski *et al.* 2007) which could be found in severe cases of closed-pyometra. A significant up-regulation of endometrial cyclooxygenase-2 gene is reported in bitches with pyometra (Silva *et al.* 2009). Recently, we have reported upregulation of IL-6, IL-8, COX-2 and PGFS in the pyometra bitches associated with endometrial atrophy (Singh *et al.* 2018). From this study, it is concluded that serum PGFM is elevated in SIRS+ canine pyometra and can aid in disease monitoring along with routine serum biochemistry. Thus, serum PGFM may serve as putative prognostic indicator for canine pyometra.

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