SOME ACUTE PHASE PROTEINS AND BIOCHEMISTRY PARAMETERS OF FEMALE DOGS AFFECTED WITH PYOMETRA

ABSTRACT

Canine pyometra is a common reproductive disorder of intact diestrual bitches. The disease is associated with inflammation and infection of the uterus leading to generalized illness. The varied reactions of the host to infection, inflammation, or trauma are collectively known as the acute-phase response (APR) and encompass a wide range of pathophysiological responses. One of these systemic responses to disease is an increase in the production by the liver of a number of serum proteins, which are known collectively as the acute-phase proteins (APP). The most important APPs in dogs are C-reactive protein (CRP) and serum amyloid A (SAA). When diagnosed with pyometra, the preferred and most effective treatment is ovariohysterectomy, a procedure that in itself causes systemic inflammation due to the tissue trauma caused by surgery. The purpose of this study was to identify the effect pyometra and ovariohysterectomy have on acute phase proteins and routinely measured biochemistry variables in serum of affected bitches. We achieved this by evaluating concentrations of CRP and SAA, as well of some biochemistry parameters in serum before and after ovariohysterectomy in bitches with pyometra. Blood samples were collected from the cephalic vein immediately prior to and 24h, 72h and 168 hours after initiation of surgery. The levels of CRP, SAA and some biochemistry parameters - total protein (TP), albumin (ALB), globulin (GLOB), as well as activity of some enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKLP) and lactate dehydrogenase (LDH) were determined

Aims: he aim of this study was to investigate the impact of pyometra and its surgical treatment, ovariohysterectomy, on acute phase proteins, specifically C-reactive protein (CRP) and serum amyloid A (SAA), as well as on routine serum biochemistry variables in affected bitches. By evaluating the concentrations of CRP and SAA, alongside key biochemistry parameters before and after surgery, this research seeks to elucidate the physiological and biochemical responses to both the disease and its treatment, providing insight into the systemic effects of pyometra and the surgical stress response in dogs.

Study design: The study design involved a cohort of twenty-seven female dogs, divided into two groups: seventeen clinically healthy intact female dogs serving as the control group, which were examined for routine health care or vaccinations, and ten female dogs diagnosed with pyometra at the Faculty of Veterinary Medicine, University of Sarajevo. The diagnosis of pyometra was made based on case history, clinical signs, ultrasonography, and confirmed by gross examination of the pus-filled uterus during and after ovariohysterectomy. The surgical procedure was performed using a standard mid-line approach under general inhalation anesthesia, with no post-operative complications reported.

Place and Duration of Study: Faculty of Veterinary Medicine, University of Sarajevo, between June 2012 and September 2012.

Methodology: Blood samples were collected from the distal cephalic vein into serum separating tubes (Vacutainer SST; Becton Dickinson, USA) for CRP and SAA measurement and serum biochemical evaluation just before the ovariohysterectomy (base values) as well as after 24 hours (day one), 72 hours (day three) and 168 hours (day seven). Blood samples from control dogs were collected only once.

Serum samples were prepared by centrifugation (1.500 × g for 10 min) and stored in plain micro tubes (Eppendorf, Germany) at −20°C prior to analysis. Serum CRP concentrations in the dogs were measured with ELISA microplate reader (Anthos 2001 ELISA-reader, Anthos Mikrosysteme, Krefeld, Germany) using a commercial canine CRP enzyme-linked immunosorbent assay (ELISA) kit (TrideltaPhaseTM Range CRP Assay, Tridelta Development Limited, County Kildare, Ireland). For SAA, the analyses were performed using also a commercially available ELISA kit (TrideltaPhaseTM Range SAA Assay, Tridelta Development Limited, County Kildare, Ireland). Total protein (TP), albumin (ALB), globulin (GLOB) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP) and lactate dehydrogenase (LDH) activities were determined by using standard procedures and slides by Catalyst Dx Chemistry Analyzer, Idexx.

Statistical analyses were performed using the SPSS software package (for Windows, Version 11.5, SPSS Inc, USA). All data are shown as mean ± standard deviation. The results were analyzed using the independent t-test. A P-value less than 0.05 was considered statistically significant.

Results: Our results indicated significantly higher concentrations of CRP and SAA in dogs with pyometra throughout the study period compared to the control group, with CRP concentrations rising up to 15-fold and SAA up to 10-fold. Notably, these increases in CRP and SAA concentrations were mostly observed before surgery, with only limited additional increases post-surgery. This suggests that while ovariohysterectomy induces a proinflammatory response, it does not significantly escalate the levels of these proteins beyond the initial disease impact. The variability observed in CRP and SAA levels points to potential interindividual differences, impacting their diagnostic value. However, on the last postoperative day, the significantly elevated levels of both proteins in dogs with pyometra suggest a persistent, though less severe, inflammatory process, with a subsequent decline as homeostasis was gradually restored.
Conclusion: The role of the acute phase response is to limit inflammation, remove the damaging factor and restore homeostasis. The systemic effects of pyometra are reflected by acute phase proteins and several biochemistry parameters.

Keywords: pyometra, C-reactive protein, serum amyloid A, biochemistry parameters, dogs)

1. INTRODUCTION

Pyometra is a reproductive disease that usually occurs during diestrus in intact adult female dogs. The disease affects on average 19% of all intact female dogs before 10 years of age (Hagman, 2017). The exact etiology is unknown; however the repeated and prolonged response to estrogen followed by long intervals of progesterone dominance in the intact female dogs leads to hormonally mediated changes in the endometrium. The endometrium changes when impacted by bacterial infiltration (Reusche et al., 2018). Pyometra is a potentially life-threatening disease associated with a variety of clinical and laboratory findings. Clinical symptoms are well described and derive from the site of infection (purulent vaginal discharge, abdominal pain) and more systemic effects (lethargy, depression, anorexia, polyuria, polydipsia and vomiting) (Gibson et al., 2013).

The acute phase response (APR) is a nonspecific inflammatory reaction of the host that occurs shortly after any tissue injury e.g., surgery, trauma, infection, or neoplasia. The main feature of APR is the production of acute phase proteins (APP) in the liver. In dogs, C-reactive protein (CRP) and Serum amyloid A (SAA) are major acute phase proteins showing significant increases in concentration as a result of systemic inflammation. CRP was first described by Tillett and Francis (Tillett and Francis, 1930) and originally named for its ability to bind the C polysaccharide of Streptococcus pneumoniae. CRP is mostly synthesized by hepatocytes. The serum CRP level is widely used as a non-specific marker for inflammation of many types. After an acute stimulus, the plasma concentration of CRP rises within 6 hours, reaching a peak at about 50 hours (Lelubre et al., 2013). The APR profile of SAA protein levels in blood is similar to that described for CRP, rising as much as 1000-fold within the initial 24 hours and then returning to very low levels as the event resolves.

The preferred and most effective treatment for pyometra is ovariohysterectomy (OVH), a procedure that in itself causes systemic inflammation due to the tissue trauma caused by surgery (Dąbrowski et al., 2009).

The systemic effects of pyometra are reflected by several laboratory parameters. The most characteristic alteration is an inflammatory leukogram with marked elevation of the total white blood cell count (WBC) and usually a regenerative left

shift in the differentiated WBC count (Fransson, 2003). Significant increase in serum transaminases and phosphatase has been reported earlier in female dogs affected with pyometra (Gupta et al., 2013).

The aim of this study was to identify the effect pyometra and ovariohysterectomy have on some acute phase proteins, as well on some biochemistry parameters in serum of affected female dogs. We achieved this by evaluating concentrations of some acute phase proteins and biochemistry parameters in serum before and after ovariohysterectomy in female dogs with pyometra.

2. MATERIAL AND METHODS

Twenty-seven female dogs were included in the study. Seventeen clinically healthy intact female dogs examined for routine health care or vaccinations were used as a control group. Ten female dogs presented for treatment of pyometra were clinically examined and diagnosed at the Faculty of Veterinary Medicine, University of Sarajevo. The presumptive clinical diagnosis was based on case history, clinical signs and ultrasonography. The diagnosis was verified by gross examination of a pus-filled uterus during and after the ovariohysterectomy. Ovariohysterectomy was performed as per the standard technique through a mid-line approach while dogs were under general inhalation anesthesia. No post-operative complications were recorded.

Blood samples were collected from the distal cephalic vein into serum separating tubes (Vacutainer SST; Becton Dickinson, USA) for CRP and SAA measurement and serum biochemical evaluation just before the ovariohysterectomy (base values) as well as after 24 hours (day one), 72 hours (day three) and 168 hours (day seven). Blood samples from control dogs were collected only once. Serum samples were prepared by centrifugation (1.500 × g for 10 min) and stored in plain micro tubes (Eppendorf, Germany) at −20°C prior to analysis. Serum CRP concentrations in the dogs were measured with ELISA microplate reader (Anthos 2001 ELISA-reader, Anthos Mikrosysteme, Krefeld, Germany) using a commercial canine CRP enzyme-linked immunosorbent assay (ELISA) kit (TrideltaPhaseTM Range CRP Assay, Tridelta Development Limited, County Kildare, Ireland). For SAA, the analyses were performed using also a commercially available ELISA kit (TrideltaPhaseTM Range SAA Assay, Tridelta Development Limited, County Kildare, Ireland). Total protein (TP), albumin (ALB), globulin (GLOB) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP) and lactate dehydrogenase (LDH) activities were determined by using standard procedures and slides by Catalyst Dx Chemistry Analyzer, Idexx.

Statistical analyses were performed using the SPSS software package (for Windows, Version 11.5, SPSS Inc, USA). All data are shown as mean ± standard deviation. The results were analyzed using the independent t-test. A P-value less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

We investigated CRP and SAA concentrations and values of some biochemistry parameters in seventeen clinically healthy female dogs (control group) and ten female dogs affected with pyometra. The ages of the dogs in control group were between one and three years; mean age was 1.8 years. The ages of the dogs with pyometra were between three to 14 years; mean age was 7.6 years. The mean values of investigated parameters of both groups of dogs and differences between them, as well as differences during pre (Day 0) and post-ovariohysterectomy (Day 1, Day 3 and Day 7) are shown in Table 1.

Table 1. Mean values of some APP and biochemical parameters in healthy dogs and pre and post-OVH female dogs affected with pyometra

PARAMETER	CONTROL GROUP	FEMALE DOGS WITH PYOMETRA			
		Day 0	Day 1	Day 3	Day 7
CRP (µg/mL)	7.48 ± 4.82a	143,00±75,88b	201,72±55,55b	76,49±20,68c	34,33±20,26d
SAA (µg/mL)	12,42±11,33a	176,59±95,89b	183,59±128,09b	124,30±77,95b	101,44±68,38b
ALB (g/L)	28,25 ±0,5b	25,30±2,54a	24,44±2,35a	24,00±2,75a	23,50±2,37a
GLOB (g/L)	31±2,94cd	40,00±6,06ae	36,78±7,89ad	44,20±5,51be	47,10±4,04b

TP (g/L)	59,75±2,63a	66,50±5,38b	61,70±5,03a	68,30±3,71bc	70,60±3,34c
ALT (IU/L)	20,00±18,69ab	29,10±14,93a	11,20±6,44a	39,90±7,19b	40,80±6,60b
AST (IU/L)	20,25±16,68ab	9,90±10,17a	10,70±9,30a	25,30±11,00b	17,20±5,33b
ALKP (IU/L)	66,5±13,48a	143,80±14,15b	211,60±18,0d	232,50±23,31e	193,30±15,06c
LDH (IU/L)	91,75±31,34b	774,50±89,28e	56,17±10,40a	166,67±14,69c	212,83±36,95d

Note: CRP – C-reactive protein; SAA - Serum amyloid A; ALB – Albumin; GLOB – Globulin; TP - Total protein; ALT - Alanine aminotransferase; AST - Aspartate aminotransferase; ALKP - Alkaline phosphatase; LDH - Lactate dehydrogenase; Different letters in each section show significant difference

The role of the acute phase response is to limit inflammation, remove the damaging factor and restore homeostasis (Karlsson et al., 2012). The systemic effects of pyometra are reflected by acute phase proteins and several biochemistry parameters.

Our results showed higher concentrations of CRP and SAA in dogs with pyometra during investigated period compared to control group. CRP concentration in our study rised up to 15-fold in reponse to pyometra; SAA rised up to 10-fold compared to control group. Elevated concentrations of CRP and SAA before surgery in dogs with pyometra, only resulted in limited, additional increases post-surgery. The increase in serum CRP and SAA concentrations has been attributed to an increased production of proinflammatory cytokines due to ovariohysterectomy. However, we have not recorded significantly higher concentrations of investigated proteins due to tissue discontinuity and local inflammation provoked by surgical trauma. Based on measure of variability (standard deviation) we may presume an interindividual variability which could be detrimental to the diagnostic value of CRP an SAA. Significantly increased concentrations of CRP and SAA in dogs with pyometra compared to control group of dogs have been demonstrated on the last postoperative day, indicating a persisting although less severe inflammatory process. Subsequent decline in their concentration was evident as the homeostasis was gradually re-established during progression of normal postoperative healing. CRP and SAA could be useful in differentiating female dogs with pyometra from healthy dogs.

As shown in Table 1. there was neither variation in albumin concentration between preoperative period (Day 0) and postoperative period (Day 1, Day 3, Day 7) (Table 1.). However, values obtained were slightly lower than reference range for albumin (26–40 g/L) (Aiello and Moses, 2016) and significantly lower than albumin concentrations measured in control group of dogs. Lower albumin concentrations are probably result of the effects of inflammation. Albumin, as one of the negative acute phase proteins in dogs decreases in response to inflammation. The mechanism by which their concentrations decrease is likely multifactorial, including decreased production by the liver in response to inflammatory cytokines, and possibly increased loss or increased proteolysis (Eckersall, 2008). Reduced production of albumin allows greater increase in the amount of amino acids available for positive acute phase proteins production, according to Paltrinieri (2008). Our results may imply that female dogs were already circulatory affected due to pyometra, since the inflammatory response causes increased capillary vascular permeability and development of hypotension.

Globulin concentrations differed significantly between healthy dogs and dogs with pyometra, but also between Day 0 and Day 7 (Table 1.). Hyperglobinemia in pyometra may occure due to dehydration and chronic antigenic stimulation of the immune system (Baithalu et al., 2010).

Total protein concentration decreased significantly after surgical procedure (Day 1). Total serum protein may be tied to a higher surgical stress, like albumin. Similar findings were reported by Munteanu et al. (2017). Their study in humans showed lower serum albumin and total serum protein values in the first postoperative day.

ALT, AST, ALP and LDH are the liver enzymes used clinically to evaluate the presence of hepatic disease. Measurement of increased serum concentrations of these substances may suggest hepato–biliary disease. According to Schäfer-Somi (2015) when ALT is decreased, about 60% of the female dogs with pyometra have higher AST levels. In our study, activity of ALT was within the reference range (8,2–57 U/L) (Aiello and Moses, 2016) and did not differ from values obtained from the control group. However, as shown in Table 1., the lowest ALT activity was determined on Day 0 (29,10±14,93 U/L) and Day 1 (11,20±6,44 U/L), while the highest level of activity was recorded on Day 7 (40,80±6,60 U/L). Increased ALT activity can reflect hepatocellular damage due to toxaemia or diminished hepatic circulation and cellular hypoxia due to dehydration (Baithalu et al., 2010).

The average serum level of aspartate aminotransferase measured in dogs with pyometra measured on Day 3 was the highest during the observed period. However, levels of AST activity were within the reference range during examined period (8,9–49 U/L) (Aiello and Moses, 2016) and did not differ significantly from control group of dogs. According to Schäfer-Somi (2015) prolonged pyometra may induce increased level of AST activity. It is possible that our findings indicate an acute process.

Activity of ALKP exceeded the reference range (10,6–101 U/L) (Aiello and Moses, 2016); at the same time, ALKP differ significantly from values recorded in control group and also during examined period (Table 1.). The reason for high levels of ALKP activity might be presense of toxemia. In female dogs diagnosed with pyometra intrahepatic cholestasis has been documented (Fransson, 2003) and presents reasonable explanation for the increases seen in ALKP.

Increased activity of LDH was recorded on Day 0, moreover, activity of this enzyme differed significantly during investigated period. Increase of LDH activity in female dogs with pyometra was reported by Yoon and associates (2017). Lactate dehydrogenase is a cytoplasmic enzyme with H (heart) and M (muscle) subunits forming LD isoenzymes (LD 1-5). Both these isoenzymes have several additional sources (erythrocytes, brain, kidneys, pancreas), which makes the interpretation of results difficult. According to Fransson (2003) LDH is often elevated in pyometra cases, suggesting that the elevated levels of LDH more likely is derived from muscle breakdown than hepatocellular insult. This suggestion was based on the observation of increases in the muscular enzyme creatine kinase (CK) and decreased activity of ALT indicating that no hepatocellular damage has occurred.

4. CONCLUSION

the systemic effects of pyometra and its treatment through ovariohysterectomy are evident in the altered levels of acute phase proteins and biochemistry parameters. These changes reflect the body's complex response to infection, inflammation, and surgical stress. CRP and SAA, in particular, emerge as valuable biomarkers for differentiating between healthy dogs and those affected by pyometra. The study underscores the importance of a comprehensive approach to diagnosing and managing pyometra, taking into account the wide array of physiological changes it induces.

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