Predictive markers and risk factors in canine and feline pyometra under Egyptian conditions

Zienab Abdelbaset *, Gamal A. Sosa, Alaa Abdel-Ghaffar, Mohamed M.M. Kandiel

Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Egypt

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ABSTRACT

Pyometra is the most prevalent reproductive disorder threatens dogs and cats’ life. This study aimed to address the relation between pyometra, and oxidative stress and hormonal milieu. Bitches (n=200) and queen (n=281) admitted to veterinary clinics from which ten animals per each were pyometric. Health examination, ultrasound investigation and blood sampling were accomplished, besides histopathology of uterus in animals underwent elective ovariohysterectomy. The separated sera were assayed for inflammatory marker (C-reactive protein; CRP), oxidative stress biomarkers (glutathione peroxidase (GPX), catalase (CAT), total antioxidant capacity (TAC), nitric oxide (NO), superoxide dismutase (SOD), lipid peroxidation (MDA)), and hormones (progesterone (P4), estrogen (E2)). Animals with pyometra showed fever, polydipsia, polyuria, vomiting and purulent vulvar discharge. Ultrasound examination revealed the presence of anechoic fluid in the uterus. A significant increase in CRP (P< 0.01 and 0.05), NO (P< 0.05), MDA (P<0.01 and 0.005), P4 (P< 0.05 and P< 0.0001), and a decrease in SOD (P< 0.0005 and 0.005), CAT (P< 0.05), and TAC (P< 0.05), in bitches and queens, respectively. Histopathology revealed a suppurative inflammation and heavy leucocytic infiltration in all uterine layers, and severe degenerative changes in endometrial glands with neutrophil infiltrated homogeneous eosinophilic substances filled the lumen. The ultrasound morphometric measures (uterine diameter, and wall thickness, and lumen and wall echo-pattern) were positively correlated with CRP and NO, and negatively correlated with TAC. In conclusion, pyometra greatly influenced the health status and reproductive efficiency of bitches and queens through altering oxidative defense mechanism.

1. INTRODUCTION

Pyometra in pet animals (dogs and cats) is a serious problem affects their life. About 20% of intact dogs experience pyometra by the age of ten years (Hagman et al., 2014). Pyometra is less common in cats than bitches since they are an induced ovulatory, however, cats whose owners do not usually go for elective neutering commonly suffer from this disorder at an age beyond 5-7 years of age (Hollinshead and Krekeler, 2016). They also recorded a high incidence of pyometra in cats is observed at diestrus or pseudopregnancy, during progesterone dominance that lasts approximately 40 days. There are similarities in clinical symptoms, etiology and diagnostic methods of pyometra in dogs and cats, with progesterone exposure predisposing to uterine bacterial infection (Hagman et al., 2014). Progesterone causes hyperplasia of the endometrium increase the secretory activity of the endometrial glands especially of the endometrial glands leading to and (Lawler and Johnston, 1995). Bacterial invasion of the uterus generates systemic inflammatory response syndrome (an exaggerated body defense response to harmful stressor) in nearly 60% of cases (Karlsson et al., 2012). C-reactive protein (CRP) is a classical acute phase protein appears in high concentration during early stage of any infection, so its level can be used as a prognostic marker for pyometra (Hillström et al., 2014).

The clinical signs of pyometra are often vague and differ between cases. Animals suffer from pyometra show signs of health disturbace (e.g., lethargy, vomiting, polydipsia) and even animal death may occur (Hagman, 2018). Lacking vulvar discharge represents a great challenge in pyometra diagnosis. Ultrasound provides useful information aid in the diagnosis/assessment of the condition non-invasively (Rautela and Katiyar, 2019). Oxidative stress is a usual phenomenon in the body. Many disturbances/diseases occur due to oxidative stress in association with the exhaustion of antioxidant system (Amin et al., 2021). One of the most important antioxidant enzymes in the biological system is superoxide dismutase (SOD) which converts superoxide anion into hydrogen peroxide that is transformed to water by catalase and glutathione peroxidase (Rahman et al., 2006). Superoxide radicals are potent inhibitors for nitric oxide creates peroxynitrite which is a strong reactive oxygen species (ROS) resulting in hydrogen peroxide and oxygen production (Grieve and Shah, 2003). MDA level is a mirror to lipid peroxidation status in the body, and therefore it is a common marker of antioxidant status and oxidative stress (El-Bahr and El-Deeb, 2016). For authors knowledge, scarce research concerned with the inter-relationship between pyometra, oxidative stress, and the occurrence of the problem in dogs and cats. Therefore, the present study aimed to focus the lights on the alteration of oxidative status and

Correspondence to: zienabdelbaset@yahoo.com
steroid hormones (progesterone (P4) and estrogen (E2)) in pyometra (diagnosis by clinical and ultrasound examination) and the histopathological changes in bitch and queen.

2. MATERIAL AND METHODS

2.1. Animals and clinical examination

In the present study about 481 of pet animals (200 bitches and 281 queens) of mixed breeds and age (1-9 years of bitches and 1.5-8 years of queens) were examined, 20 of them showed health distress assumed pyometra (10 bitches and 10 queens). Animals were admitted to the clinic at theriogenology Dept., Fac. Vet. Med., Cairo University as well as private pet clinics in Cairo during period from June 2020 to Dec. 2021, animals were examined for health status and symptoms of pyometra include body temperature, general examination including animal demeanour, body condition according to Misk and EL-sherry (2020).

2.2. Ultrasound examination

Ultrasound (US) examination was conducted while the animal in dorsal recumbent position using a real-time B-mode ultrasound scanner (Akai AK-30 Ultrasound, China) equipped with a multifrequency micro convex transducer according to Kähn (2004) in brief, a carboxymethyl-cellulose gel was applied in the area between the two rows of mammary glands from the pubis to umbilicus. The ultrasound probe was firmly positioned against the skin of the animal in dorsal recumbent position using a real-time B-mode ultrasound scanner. The US probe proceeded cranially to visualize the uterus at the fundus of UB with probe rotation from side to side. Ultrasound was performed to evaluate uterine size, uterine wall thickness and fluid accumulation within the lumen of the uterus (Misk and EL-sherry, 2020).

2.3. Blood sampling and assessment

Non-hemolyzed blood samples were collected from animals through cephalic venipuncture into plain vacutainer tubes under aseptic precautions according to Sharif et al. (2013). Blood samples were centrifuged at 3000 rpm for 15 min and the harvested sera were stored at -20 °C according to Amin, et al. (2014), until being assayed.

2.3.1. Measurement of C-reactive protein

C-reactive protein (CRP) level was measured using the ELISA method using a commercial kit (Ichromia CRP-25, Tridelta, Ireland) according to Pepys and Hirschfield, (2003)

2.3.2. Measurement of antioxidants biomarkers

Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and catalase enzyme activity, total antioxidant capacity (TAC), nitric oxide (NO) and malondialdehyde (MDA) were measured colorimetrically using commercial kits (SD 25 21, GP 25 24, CA 25 17, TA 25 13, NO 25 33 and MD 25 29 supplied by BIO-DIAGNOSTIC, Giza, Egypt) (Nishikimi et al., 1972; Paglia and Valentine,1976; Aebi, 1984; Koracevic et al., 2001; Montgomery and Dymock, 1961; Ohkawa et al., 1979, respectively).

2.3.3. Steroid hormone assessment

Serum progesterone (P4, ng/ml) and estradiol (E2, pg./ml) concentrations were quantitatively estimated by ELISA method according to Hanita and Hanisah (2012) and Goldstein et al. (1982), respectively, using commercial ELISA kits (EIA-1561 and EL1-1254, respectively) supplied by DRG Diagnostics GmbH, Germany and Chemux BioScience, South San Francisco, USA

2.4. Histopathological examination

Small tissue specimens (1cm²) cut from uterine tissues after ovariohysterectomy were processed for histopathological examination (Maya-Pulgarin et al., 2017). Samples were fixed in 10% in neutral formalin for 72 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. The paraffin tissue block was sectioned at 5 µm thickness, stained with Hematoxylin-Eosin (H&E) and examined under a Nikon Eclipse E800 light microscope according to Anderson and Bancroft, (2002). The OMAX 9.0 MP USB digital camera (China) was used for image capture. Quantitative analysis of histopathological changes (epithelial thickness (ET), endometrial glands diameter (EGD), endometrial glands wall thickness (EGWT) and myometrium thickness (MT)) according to Floyd (2002) and uterine ultrasound parameters (uterine horn diameter (UHD), uterine wall thickness (UWT), Lumen mean gray value (LMGV) and wall mean gray value (WMGV)) according to Chou et al. (2010) were accomplished using NIH Image J (National Institutes of Health, Bethesda, Maryland, USA).

2.5. Statistical analysis

Data were computed and expressed as mean ± SEM using SPSS software (IBM® SPSS® statistics, version 23) according to Ho (2013). The incidence of pyometra in bitches and queens was calculated by chi-square (χ2) analysis. Independent-samples t-test compared between pyometric and non-pyometric animals. The correlation between CRP, TAC and NO and ultrasound parameters was verified by Spearman’s correlation coefficient. Significance level for all statistical tests was P<0.05.

3. RESULTS

3.1. Clinical examination

Animal admitted with pyometra showed signs of fever, anorexia, lethargy, vomiting and abdominal distention (Fig. 1A). In the meantime, vulvar discharge although was obvious (Fig. 1B, 1C), it was absent in some cases, especially cats. Pyometra was recorded in bitches (n=10/200, 5.0 %) and queens (n=10/281, 3.6%) of an age 5.24±1.17 and 3.8±1.21 years, respectively. The incidence rate of pyometra cases in bitches was not significantly varied than that in queens (χ2= 0.61, P= 0.44).

Fig (1): Clinical symptoms of pyometra. A: Abdominal distention (arrow) in Lolo bitch. B: Vomiting (curved arrow) and purulent vulvar discharge (notched arrow) in pit-bull bitch. C: Wet hair (arrowhead) in perineal region and around vulva in Persian queen

3.2. Ultrasound measures

In dogs and cats, uteri with pyometra appeared highly convoluted and distended with anaerobic to hypoeochic fluid
and variable wall thickness (Ultrasonogram 1). The ultrasound quantified changes in the uterus of bitches and queens suffered from pyometra is presented in table (1). There was a significant increase in UHD (P < 0.0001), UWT (P < 0.0001), LMGV (P < 0.0001) and WMGV (P < 0.0001) in pyometric bitches and queens, respectively, compared to non pyrometric group.

### Table (1): Uterine quantitative ultrasound measures in bitches and queens with pyometra

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Bitches</th>
<th>Queens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-pyrometric group</td>
<td>Pyrometric group</td>
</tr>
<tr>
<td>Uterine horn diameter (cm)</td>
<td>1.15±0.26</td>
<td>5.29±0.10** ***</td>
</tr>
<tr>
<td>Uterine wall thickness (cm)</td>
<td>0.37±0.04</td>
<td>1.41±0.15***</td>
</tr>
<tr>
<td>Lumen mean-value (pixels)</td>
<td>29.72±1.29</td>
<td>46.91±1.67****</td>
</tr>
<tr>
<td>Wall mean gray value (pixels)</td>
<td>47.48±4.79</td>
<td></td>
</tr>
</tbody>
</table>

Data (mean ± SE, n=10). ** and *** indicated significant differences at P< 0.05, 0.01 respectively.

### 3.4. Histopathological findings

In comparison with the normal uteri (Fig. 2A & 3A) of bitches and queens, respectively, the uterus in pyometra showed disturbed histological architecture characterized by severe suppurative inflammation in all layers of the uterus with heavy polymorphonuclear cellular infiltration in tunica mucosa, submucosa and muscularis. The uterine lumen was filled with pus in the form of finely granular eosinophilic and basophilic substances infiltrated with neutrophil (Fig. 2E).

Moreover, there was severe epithelial desquamation in the endometrium (Fig. 2B & 3B). The uterine submucosa showed severe degenerative changes in most of endometrial glands. The affected glands showed hyperplasia of lining epithelium with few leukocytic infiltration (Fig. 2C & 3C), and filling of the lumen with neutrophils-infiltrated homogeneous structureless eosinophilic substances (Fig. 2D). The queen’s myometrium showed severe hyaline degeneration with mild leukocytic infiltration (Fig. 3D).

Quantitative analysis of the histopathological changes (Table 4) in bitches and queens showed a significant decrease in ET (P < 0.0001, 0.001), and an increase in EGWT (P < 0.01, 0.0001), EGD (P < 0.005, 0.0001), while no marked variations were noted in MT.

### Table (2): Effect of pyometra on histopathological structure of uteri of bitches and queens

Data regarding the changes in biochemical and hormonal parameters in bitches and queens showed pyometra is presented in table (2). There was significant increase in CRP (P < 0.01, 0.05), MDA (P < 0.01, 0.005), P4/B & 0.0001, 0.0005), CAT (P < 0.05), TAC (P < 0.05), in pyometric bitch and queen, respectively. There were no marked variations in E2, GPX enzyme activity levels compared to non-pyrometric group. There was a slight (P = 0.09) decrease in E2 and E2/P4 ratio in queens showed pyometra.

The quantized ultrasound measures (UHD, UWT, LMGV and WMGV) were positively correlated with CRP (R= 0.98, 0.94, 0.93 and 0.95, respectively in bitches, and R= 0.79, 0.77, 0.78 and 0.77, respectively, in queens), NO (R= 0.97, 0.93, 0.92 and 0.91, respectively, in bitches, and R= 0.97, 0.96 and 0.98, respectively, in queens), and negatively correlated with TAC (R= 0.92, 0.87, 0.85 and 0.84, respectively, in bitches, and R= 0.77, 0.78, 0.79 and 0.77, respectively, in queens) (Table 3).

### Table (3): Effect of pyometra on hormonal and biochemical parameters in bitches and queens

**Correlation and regression coefficients in bitches and queens**

### Table (4): Effect of pyometra on histopathological structure of uterus of bitches and queens

Data (mean ± SE). **, *** indicated significant differences at P < 0.01, 0.005, 0.0001 respectively.
4. DISCUSSION

Pyometra in dogs and cats is a dangerous uterine disorder affecting their reproduction and life-as well (Hagman et al., 2014)). In the current study, there was a profound increase in CRP, NO and MDA, and a depression in SOD and CAT activities accused for the decrease in TAC. These change coincident with a disturbance in the hormonal milieu characterized by an increase in P4 levels and a decrease in P4/E2 ratio. It could be postulated that pyometra induces serious inflammatory changes and interferes with body antioxidant mechanisms which affects animals’ life.

In the current study, 5.0% (10 out 200) bitches and 3.5% (10 out of 281) queens of an age 5.24±1.17 and 3.8±1.21 years, respectively brought to gynecological clinics for examination suffered from pyometra. The incidence of dogs and cats pyometra is still not well recorded and probably under-estimated. A higher incidence (19%) of pyometra in bitch was reported by Jitpean (2015). Limited data availability and breed differences may blame for this difference (Antonov et al., 2015). On the other hand, Hagman (2018) found that pyometra was not common in queens as bitches, this might be explained by queens are induced ovulator so less progesterone dominance (Hollinshead and Krekeler, 2016). The incidence of pyometra raises in bitches > 4 years of age (Smith, 2006) and cats > 5 years (Hagman, 2018) due to repeated estrous cycles without pregnancy with changes occur in uterine wall under progesterone effect (Hagman et al., 2014). Clinical symptoms related to pyometra are not specific, except for vulvar discharge which is not usually present in dogs and very scarce in cats. Several clinical signs were observed in cases of pyometra include fever, anorexia, depression, vomiting, abdominal distention, polyuria, polydipsia, weight loss. These findings indicated the local and systemic influences of the disease. Likewise, similar findings were reported formerly (Hagman, 2018). In dogs, the presence of vulvar discharge varied from 65-77% and it may be absent in closed pyometra (Jitpean, 2015). Nevertheless, vulvar discharge can also be absent in queens due to the self-care habits (Von Reitzenstein et al., 2000). The presence of fever in pyometra may be attributed to septicemia and toxemia which causes anorexia and depression (Rautela and Katiyar, 2019). Abdominal distention was a marked sign observed in all cases suffered from pyometra due to fluid accumulation in the uterus (Von Reitzenstein et al., 2000). Polyuria and polydipsia may be attributed to endotoxins release following bacterial invasion which interfere with renal Na+ and Cl absorption leading water absorption impairment. E. coli endotoxins also block receptors of antidiuretic hormone on the collecting ducts, leading to obligatory polyuria with compensatory polydipsia (Macintire, 1994). Ultrasonography is proved as a helpful non-invasive mean in identifying uterine enlargement and differentiating pyometra from a normal pregnancy (Mattei et al., 2018). The uterus of bitches and queens suffered from pyometra was characterized by an accumulation of anechoic to hyperechoic fluid with significant increase in the value of UWD, UWT, LMGV and WMGV. This agreed with Misk and EL-sherry (2020), who noticed that uteri of pyrometric pet animals appeared distended with anechoic to hypoechoic fluid in associated with uteri wall thickness due to the presence of uterine exudates or pus (Rautela and Katiyar, 2019). C-reactive protein (CRP) is the 1st acute-phase proteins rapidly elevated at the onset of any infection or inflammation (Du Clos and Mold, 2004). It binds to several intra-cellular antigens of damages cells and pathogenic micro-organisms (Murata et al., 2004). In the present study, CRP markedly elevated in pyrometric animals, and its levels were positively correlated with the ultrasound detectable changes, indicating its diagnostic value for pyometra in pet animals. This finding agreed with Hillström et al. (2014), who noticed that CRP is an inflammatory marker, increases in cases of inflammation including pyometra cases. An inequality between the reactive oxygen species (ROS) production and their elimination is responsible for the oxidative stress (Hussain et al., 2016). Such status leads to chronic inflammation occurrence. In the present study, TAC significantly decreased in pyometra cases coincident with a decrease in SOD and CAT activities and an increase in NO and MDA levels. Former studies indicated a decrease in serum TAC levels (Amin et al., 2021) in pyrometric dairy cows, and SOD and catalase in pyrometric bitches (Rautela et al., 2018), perhaps due to an exhaustion of antioxidant system (Amin et al., 2021). SOD is considered as the 1st line of defense against ROS where it removes both intra- and extracellular superoxide radicals and decreases the danger of these radicals (Grieve and Shah, 2003). SOD protects cells during oxidative stress by breaking superoxide radicals forming hydrogen peroxide, which later dissociates to water (Powers and Jackson, 2008). NO is pro-inflammatory mediator, however, the increment in NO in pyrometric dogs and cats here might indicate its role in the pathogenesis of pyometra as the overproduction of NO induces inflammation through tissue damage and vasodilatation (Sharma et al., 2007). They also observed a significant increase in the circulating MDA in pyometra cases. MDA is a degradation byproduct from lipid peroxidation (oxidation of membrane phospholipids Similarly, Rautela and Katiyar (2019) noticed elevated MDA level in pyometra, due to the imbalance between body pro-oxidants and antioxidants mechanisms causing cellular structure damage (Nishikimi et al., 1972). Pyometra is a foremost uterine disorder mediated by prolonged exposure to P4. In the present study, P4 level
increased and the E2/P4 ratio was altered. Similarly, a substantial increase in P4 hormone in the condition of pet animals suffered from pyometra was noticed (Hollinshead and Krekeler, 2016). This might explain the occurrence of this condition in the diestrus phase due to progesterone dominance from an increased corpora lutea activity (Hollinshead and Krekeler, 2016). Quantifiable histopathological changes revealed a significant decrease in ET, and an increase in EGWT and EGID (P< 0.005, 0.0001), while no marked variations were noted in MT. The increase in the subepithelial connective tissue zone containing endometrial glands accused to the main changes in the uterine wall found here. These results agreed with Kempiest et al. (2013), who found an increase in endometrial glands size and number. Nevertheless, there was a significant decrease in epithelial thickness in pyometra cases (Rautela and Katiyar, 2019). They also detected epithelial damage due to high ROS production in association with inflammatory conditions that damages epithelial cells barrier resulting in tissue injury and severe epithelial desquamation.

5. CONCLUSION

The health status and reproductive efficiency of pyometric dogs and cats were greatly compromised by the inflammatory condition and oxidative stress. This is interrelated with the altered endocrine milieu which predisposes to the chronicity of the inflammation. This study is a step to expand the field of future diagnosis of pathophysiology of pyometra in bitches and queens.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES