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Changes in phosphorus and some biochemical parameters in cats with open and closed cervix pyometra

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ABSTRACT

Pyometra is a disease defined as a purulent inflammation of the uterus and may have multisystemic effects. In this study, the investigation of the changes in leukocytes, calcium, phosphorus, and some biochemical parameters in cats with open or closed cervix pyometra was aimed. A total number of 48 queens were enrolled in the study. Twenty three healthy queens presented in diestrus phase were classified as Group C that revelaed to the clinic for elective ovariohysterectomy. Twenty five queens constituted the pyometra group (Group PYO) which were divided into 2 subgroups whether the presence of vaginal discharge (open cervix pyometra; Group OP; n=15 and closed cervix pyometra; Group CP; n=10). The mean white blood cell (WBC) level in the Group PYO was significantly higher than in Group C ($P<0.001$). The highest WBC level was detected in the Group CP. Serum alanine aminotransferase (ALT) and total protein (TP) levels in the Group CP were higher than those detected in the Group OP ($P<0.01$) and Group C ($P<0.05$). The highest albumin (ALB) ($P<0.05$) and albumin/globulin (ALB/GLOB) ($P<0.01$) levels were determined in the Group C. Serum globulin (GLOB) levels in the Group CP and Group OP were significantly higher than those measured in the Group C ($P<0.01$). A significant rise in alkaline phosphatase (ALKP) levels in the Group PYO were detected ($P<0.01$). The highest phosphorus (PHOS) levels were identified in the Group CP ($P<0.01$). In conclusion, to the best of our knowledge, this is the first report which is detected hyperphosphatemia in the closed cervix pyometra in the queens. Besides, pyometra is a serious disease that needs to be well evaluated with laboratory tests in order to reveal its multisystemic effects.

Keywords: biochemistry, cat, phosphorus, pyometra

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Introduction

Pyometra is an acute or chronic suppurative inflammation of the uterine wall in queens. It is characterized by endometrial hyperplasia with cystic dilation of endometrial glands and accumulation of purulent exudate in the uterine lumen (Hollinshead and Krekeler, 2016). Cystic endometrial hyperplasia-pyometra complex is not very common in queens (Agudelo 2005). The disease usually develops during the luteal phase, and progesterone plays a key role for the establishment of infection with ascending opportunistic bacteria (Hagman et al., 2014). The incidence of the feline pyometra increases over five years of age (Johnston et al., 2001). However, pyometra occurrence has also been reported after the first oestrus in a five-month-old mixed breed queen (Günay Uçmak et al., 2019). Hemorrhagic and/or mucopurulent vaginal discharge is noticeable in open cervix pyometra. Besides, more severe clinical signs and abdominal enlargement frequently occur in closed cervix pyometra cases (Hagman, 2022). The common systemic signs are depression, anorexia or inappetence, vomiting, diarrhoea, listlessness, abdominal distention, polyuria and polydipsia. Pyometra affects the function of several organs,

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including marrow, liver and kidneys (Johnston et al., 2001). It usually develops a normocytic, normochromic non-regenerative anaemia and leukocytosis. In addition, serum chemistry abnormalities including hyperproteinemia, hyperglobulinemia, hypokalemia, and azotemia are presented in cases of pyometra (Nak et al., (2005).

The aim of this study is to evaluate the changes in leukocytes, calcium, phosphorus and some biochemical parameters in cats with open and closed cervix pyometra.

Materials and methods

Animals and study design: All animal procedures were carried out in accordance with the approval of the Istanbul University-Cerrahpaşa Animal Experiments Unit Ethical Committee (Approval number: 2022/42). A total number of 48 queens were enrolled in the study. Gross clinical (hearth rate, respiratory rate, body temperature and dehydration status) and gynaecological (vaginal cytology and transabdominal ultrasonography with 6.6 MHz convex transducer (Esaote Pie Medical MyLab Five Vet., Genova, GE, Italy)) examinations were revealed. Twenty three healthy queens in diestrus that presented to the clinic for ovariohysterectomy were included in control group (Group C). Twenty five queens constituted the pyometra group (Group PYO). The pyometra was treated with ovariohysterectomy. The queens in group PYO were divided into 2 groups with regard to the presence of vaginal discharge in which consisted of open cervix pyometra (Group OP; n=15) and closed cervix pyometra (Group CP; n=10). Before all queens had surgical intervention, hematological analyses were performed.

Hematology and biochemistry: Blood was drawn by the puncture of jugular vein and collected into one clot separator tube and one EDTA containing tube prior to surgery. Blood biochemistry (DRI-CHEM NX600, Fujifilm, Japan) and total blood count tests (Procyte Dx Hematology Analyzer, Idexx, USA) were performed. White blood cell (WBC), glucose (GLU), creatinin (CREA), blood urea nitrogen (BUN), BUN/CREA, total protein (TP), globulin (GLOB), albumin (ALB), ALB/GLOB, Alanine aminotransferase (ALT), Alkaline Phosphatase (ALKP), calcium (Ca) and inorganic phosphorus (PHOS) measurements were incorporated into the study.

Vaginal cytology: Vaginal smear was obtained for cytological examination of the vagina for both groups. The smears were stained with Diff-Quick stain set (ADR Group, Mediko Kimya, Istanbul, Turkey) according to the manufacturer's instructions. Slides were examined by a light microscope (Olympus CX41, Tokyo, Japan) at x 400 magnification.

Statistical analysis: Statistical analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, Illinois, USA). Normality of the data was analyzed by Shapiro Wilk test. The normally distributed data were tested by One-way ANOVA for the comparison of the groups with regard to the hematological parameters. The Pearson correlation was used to determine the relationships among the parameters evaluated. Values were given as mean ± standard error of the mean (SEM). The significance level was accepted as $P<0.05$.

Results

The mean ages and SEM in group C and group PYO were 4±0.45 years and 5±0.68 years; respectively ($P>0.05$). Vaginal cytology smears of all queens included into the study had intermediate cells on the slide while polymorphonuclear neutrophils were dense in group PYO. Also, background of the smear slides were not clean in queens with open cervix pyometra. In group PYO, enlarged uterine horns and uterine wall were observed in abdominal ultrasonography. Also, uterine lumen was visualized as fulfilled with pus in pyometra affected queens. The corpora lutea were observed in the ovaries of the queens during ovariohysterectomy. The mean values and SEM of hematological parameters and their significances related to the groups were presented in Table 1, whereas the relationships among the evaluated parameters were given in Table 2. Accordingly, positive correlations were observed between PHOS and ALKP ($P<0.01$, $r=0.423$), CA and CREA ($P<0.05$, $r=0.341$), GLOB and TP ($P<0.001$, $r=0.872$), BUN and CREA ($P<0.001$, $r= 0.511$).

Table 1. The concentration of white blood cell and biochemistry profile of healthy (Group H), open (Group OP) and closed-cervix pyometra (Group CP) groups.

	Group C (n=23)	Group OP (n=15)	Group CP (n=10)	Sig.
WBC (10^9/L)	8.96±0.75^a	23.42±4.34^b	41.95±8.40^c	P<0.001
GLU (mg/dL)	120.69±7.00	120.75±7.63	151.60±21.19	ns
CREA (mg/dL)	1.40±0.07	1.34±0.07	1.34±0.31	ns
BUN (mg/dL)	20.58±1.28	20.62±2.25	18.80±3.01	ns
BUN/CREA (mg/dL)	15.95±0.84	16.37±2.39	15.60±1.60	ns
TP (g/dL)	7.50±0.12^a	7.98±0.18^{ab}	8.03±0.22^b	P<0.05
ALB (g/dL)	3.23±0.06^a	3.00±0.06^b	2.98±0.07^b	P<0.05
GLOB (g/dL)	4.27±0.11^a	4.95±0.20^b	4.88±0.23^b	P<0.01
ALB/GLOB	0.76±0.03^a	0.62±0.02^b	0.62±0.03^b	P<0.01
ALT (U/L)	63.86±7.36^a	78.37±21.90^a	154.10±35.83^b	P<0.01
ALKP (U/L)	30.56±2.99^b	50.39±5.10^a	58.10±10.69^a	P<0.01
Ca (mg/dL)	9.87±0.18	9.75±0.12	9.92±0.24	ns
PHOS (mg/dL)	4.95±0.18^a	4.66±0.20^a	5.95±0.39^b	P<0.01

a, b, c : Different letters indicate the significance. ALB: Albumin, ALKP: Alkaline Phosphatase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, Ca: Calcium, CREA: Creatinin, GLOB: Globulin, GLU: Glucose, PHOS: Phosphorus, TP: Total protein, WBC: White blood cell. ns: P>0.05

Table 2. Pearson correlation assessment of the parameters related to the haematology and biochemistry both in open and closed cervix pyometra.

	WBC	GLU	CREA	BUN	BUN/ CREA	TP	ALB	GLOB	ALB/ GLOB	ALT	ALKP	CA	PHOS
WBC	1	0.240 ns	0.150 ns	0.189 ns	0.120 ns	0.144 ns	-0.394 **	0.249 ns	-0.353 *	0.088 ns	-0.177 ns	0.048 ns	0.177 ns
GLU	0.240 ns	1	0.245 ns	0.199 ns	0.011 ns	0.184 ns	-0.020 ns	0.234 ns	-0.187 ns	-0.04 ns	-0.238 ns	0.035 ns	-0.249 ns
CREA	0.150 ns	0.245 ns	1	0.511 ***	-0.391 **	0.022 ns	0.004 ns	0.042 ns	-0.083 ns	-0.173 ns	-0.126 ns	0.341 *	0.037 ns
BUN	0.189 ns	0.199 ns	0.511 ***	1	0.403 **	-0.069 ns	0.048 ns	-0.068 ns	0.036 ns	-0.187 ns	-0.269 ns	0.167 ns	-0.094 ns
BUN/ CREA	0.120 ns	0.011 ns	-0.391 **	0.403 **	1	-0.131 ns	0.078 ns	-0.091 ns	0.022 ns	-0.052 ns	-0.051 ns	-0.176 ns	-0.066 ns
TP	0.144 ns	0.184 ns	0.022 ns	-0.069 ns	-0.131 ns	1	0.033 ns	0.872 ***	-0.616 ***	0.044 ns	0.153 ns	0.140 ns	0.199 ns
ALB	-0.394 **	-0.02 ns	0.004 ns	0.048 ns	-0.078 ns	0.033 ns	1	-0.352 *	0.676 ***	0.041 ns	0.184 ns	0.196 ns	-0.136 ns
GLOB	0.249 ns	0.234 ns	0.042 ns	-0.068 ns	-0.091 ns	0.872 ***	-0.352 *	1	-0.866 ***	0.004 ns	0.79 ns	0.065 ns	0.181 ns
ALB/ GLOB	-0.353 *	-0.187 ns	-0.083 ns	0.036 ns	0.022 ns	-0.616 ***	0.676 ***	-0.866 ***	1	0.001 ns	0.010 ns	0.014 ns	-0.270 ns
ALT	0.088 ns	-0.040 ns	-0.173 ns	-0.187 ns	-0.052 ns	0.044 ns	0.041 ns	0.004 ns	0.001 ns	1	0,236 ns	0,005 ns	0,184 ns
ALKP	-0.177 ns	-0.238 ns	-0.126 ns	-0.269 ns	-0.051 ns	0.153 ns	0.184 ns	0.079 ns	0.010 ns	0.236 ns	1	0.036 ns	0.423 **
CA	0.048 ns	0.035 ns	0.341 *	0.167 ns	-0.176 ns	0.140 ns	0.196 ns	0.065 ns	0.014 ns	0.005 ns	0.036 ns	1	-0.189 ns
PHOS	0.177 ns	-0.249 ns	0.037 ns	-0.094 ns	-0.066 ns	0.199 ns	-0.136 ns	0.181 ns	-0.270 ns	0.184 ns	0.423 **	-0.189 ns	1

ALB: Albumin, ALKP: Alkaline Phosphatase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, Ca: Calcium, CREA: Creatinin, GLOB: Globulin, GLU: Glucose, PHOS: Phosphorus, TP: Total protein, WBC: White blood cell. ns: P>0.05 , *: P<0.05 , **: P<0.01 , ***: P<0.001

Discussion

Pyometra which is an inflammatory disorder of the uterus, usually occurs over 5 years of age in queens (Hagman et al., 2014). Although the significant differences were not observed related to the ages of the queens in either group, the mean age of the group PYO was at the verge age which was reported before (Hagman et al., 2014).

Hormones and bacteria are involved in the disease development (Hagman 2022). The diagnosis is based on case history, clinical signs and findings on physical examination, hematology and biochemistry laboratory tests, and diagnostic imaging techniques (especially B-mode ultrasonography) (Keshavprasad et al., 2023). The same diagnostic methods with the previous report (Keshavprasad et al., 2023) were used to determine the pyometra in this study.

Most of the queens with pyometra show abnormal WBC which is frequently characterized by leukocytosis (Agudelo, 2005). As previously reported, the mean WBC was significantly higher in group PYO than in healthy queens in this study. While increased total leukocyte counts may be found in the cat with open-cervix pyometra, normal leukocyte counts may also be encountered (Hollinstead and Krekeler, 2016). In contrast with the researchers' report, definite rise in WBC was also observed in group OP as compared to the Group C, in this study. The total leukocyte count is generally increased in the cat with closed-cervix pyometra, usually exceeding 30x109/L (Kenney et al., 1987). Similarly, the highest WBC levels were detected in Group CP in the study. Absolute leukocytosis in closed cervix pyometra is thought to be depended on the severity of the disease and septicemia.

The renal dysfunction may secondarily be developed due to the bacterial endotoxemia, dehydration and azotemia (Alaçam, 1998). Unlike bitches, queens have significantly less pyometra-related kidney damage (Hollinstead and Krekeler, 2016). It was stated that a high CREA concentration was determined in 12% of the cats with pyometra (Kenney et al., 1987). Nak et al. (2005) reported that there was no difference between pyometra and healthy queens in terms of BUN and CREA values ($P>0.05$). In this study, GLU, BUN and CREA levels were not significantly different related to the presence of pyometra ($P>0.05$). It is thought that the similar results have been obtained in accordance with the previous studies, since the lower incidence of renal failure is more common due to pyometra the in female cats.

Enginler et al. (2014) detected that TP concentrations showed a significant increase in pyometra group than those measured in healthy controls. In this study, the mean TP level of the queens with closed cervix pyometra was significantly higher as compared to the queens with the open cervix pyometra and the control group. The finding might be explained by the suggestion of Borresen and Skreden (1980) who found the similar results and indicated that plasma protein levels in pyometra cases has changed as a result of acute phase reactions.

Albumin is considered to be a negative acute phase protein as the serum concentrations decrease in inflammation and/or infection (Hagman 2012). Fantoni et al. (1999) stated that vascular permeability increase due to the sepsis and endotoxemia which subsequently led to loss of protein. Vilhena et al. (2018) have detected the decreased ALB concentrations in queens with pyometra. In accordance with Vilhena et al. (2018), serum ALB concentration was significantly higher in healthy queens than those detected in the Group PYO. The similar results may have been obtained due to the decreased production and increased loss of this protein during systemic inflammation (Fantoni et al., 1999; Werner and Turnwald, 1999; Hagman, 2012).

In feline pyometra, hyperglobulinemia is usually one of the mild changes in serum biochemistry (Hollinstead and Krekeler 2016). Johnson (1994) reported that hyperglobulinaemia can be present in 30 -60% of the pyometra cases in cats. In consistent with the previous reports, the mean levels of GLOB in both pyometra groups (group CP and group OP) were significantly higher than group C in this study. Alpha-2 or beta globulins increase in the acute inflammation, and gamma globulins increase in the chronic inflammation (Comazzi et al., 2004). This may explain why hyperglobulinemia is detected in both open and closed cervix pyometra.

Pyometra can cause liver disarrangement (Nak et al., 2005). In a group of 183 cats with pyometra, only 7% with high ALT activity was reported (Kenney et al., 1987). Although the ALT obtained in this study was not at a low percentage as in Kenney's report, the ALT level was also found to be high in the closed cervix pyometra group. Increase of ALT levels may be due to the hepatocellular damage caused by septicemia, diminished hepatic circulation and cellular hypoxia in the dehydrated cats as Nak (1999) reported.

The most sensitive tests for hepatic diseases are ALT and ALKP which rise especially in the presence of sepsis and dehydration (Hollinstead and Krekeler, 2016). Increase in ALKP is often associated with hepatobiliary obstruction, with a very high sensitivity in the dog in comparison to the cat (Comazzi et al., 2004). In both open and closed cervix pyometra cases, elevated ALKP levels were observed in this study. Also, the queens with closed cervix pyometra had the highest ALKP level. These changes reflect hepato-cellular damage in response to toxemia, sepsis or decreased hepatic perfusion due to the dehydration as Verstegen et al. (2008) stated.

It was reported that significant differences were not observed, when the serum calcium levels of bitches suffered from pyometra were compared with the healthy ones (Asheim, 1964). Similar to the previous report (Asheim, 1964), the mean Ca level in Group PYO was not significantly different than those measured in the Group C in this study. King et al. (2007) detected mild correlation between plasma creatinine concentration and plasma calcium levels in cats with chronic kidney disease. Similarly, creatinine concentration was significantly but not highly correlated with calcium in this study ($P<0.05$ and $r=0.341$). Pyometra can cause polyuria which shows the reduction of renal concentrating ability. A similar renal dysfunction occurs in conjunction with potassium depletion and with hypercalcemia (Asheim, 1964). Considering the results of our study, it is thought that not only calcium and creatinine but also potassium should be evaluated for kidney damage/ dysfunction.

Verstegen et al. (2008) observed elevated serum ALKP levels in approximately 50–75% of pyometra cases in bitches. Also, high circulating PHOS levels lead to hepatic dysfunction by considerably lowered initial blood sugar and the glycogen content of the liver (Althausen and Thoenes, 1932). In this study, serum ALKP levels were strongly correlated with serum PHOS levels ($P<0.01$; $r=0.423$). This positive relationship

could be explained by the effect of both parameters on liver function as the researchers previously reported (Althausen and Thoenes, 1932; Verstegen et al., 2008).

Chung et al. (2003) suggested that lower serum phosphorus levels in patients who recovered from hepatic failure may be associated with recovery of hepatic function. Also, hyperphosphatemia had been associated with the lower survival rate and increased morbidity in cats with chronic kidney disease (King et al., 2007). Koo et al. (2011) reported the serum PHOS levels within the reference ranges in two young dogs with closed-cervix pyometra. Unlike the above mentioned research (Koo et al. 2011), the highest inorganic PHOS levels were detected in Group CP in this study. Particularly in cases of closed cervix pyometra, severe systemic clinical signs are often observed as renal and hepatic dysfunction (Kurtzler et al., 2012). The hyperphosphatemia in queens with closed cervix pyometra could be explained by the more frequent occurrence of renal and hepatic dysfunction in pyometra cases (Kurtzler et al, 2012) and negative impact of serum PHOS levels on the hepatic and renal function (Chung et al., 2003; King et al., 2007). To the best of our knowledge, this is the first report which is detected hyperphosphatemia in closed cervix pyometra in queens.

In conclusion, pyometra is a serious disease that needs to be well evaluated with laboratory tests in order to reveal its multi-systemic effects. Although transabdominal ultrasonography is a golden standart to diagnose the pyometra, hemogram and biochemistry tests are essential to diagnose the multi-systemic effects of this illness. Besides, the presence of hyperphosphatemia could be predictive to reflect the hepatorenal dysfunction in closed cervix pyometra cases of queens.

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