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Original article

Evaluation of calcium, phosphorus and some biochemical parameters in dogs with open and closed cervix pyometra

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Abstract

The aim of this study was to evaluate the changes in calcium, phosphorus and some biochemical parameters in dogs with open and closed cervix pyometra, which was then compared with a control group. A total of 62 bitches of age group 5-10 years old irrespective of breed were enrolled into the study. Control group consisted of 22 bitches which were clinically healthy and in luteal phase of the estrus cycle. On clinical examination, pyometra was diagnosed in 40 bitches while 23 out of 40 bitches had open-cervix pyometra and 17 of 40 bitches had closed-cervix pyometra. Evaluation of haematological changes revealed that there was increased white blood cell (WBC), total protein (TP), globulin, alkaline phosphatase (ALKP) while decreased glucose (Glu), albumin (Alb) and albümin globulin ratio in pyometra cases ($p<0.001$). The highest WBC and ALKP levels were detected in closed-cervix pyometra group ($p<0.001$). Calcium (Ca^{+2}) levels had significant rise in open-cervix pyometra cases ($p<0.05$) while phosphorus (Phos) levels had tend to increase in closed-cervix pyometra cases ($p=0.08$). Also, Ca was directly correlated with Glu, Alb and TP while inversely correlated with WBC and blood urea nitrogen/creatinine (BUN/Crea). However, Phos was significantly associated with BUN and Crea ($p<0.001$; $R=0.915$ and $R=0.860$, respectively). As a result of this study, it was observed that the increased Ca levels in open-cervix cases and the elevated Phos levels in closed-cervix cases, were associated with the biochemical parameters related to hepatic and renal dysfunction in dogs with pyometra.

Keywords: biochemical alterations, calcium, dog, phosphorus, pyometra

Introduction

Pyometra is a common inflammatory disease resulting in the accumulation of pus inside the uterus of intact bitches which causes systemic illness (Ahuja et al. 2019, Rautela and Katiyar 2019). The age of occurrence for canine pyometra ranges from 4 months to 16 years (Jisna and Sivaprasad 2020). Pyometra is categorized according to cervical patency. Open cervix pyometra is characterized by the outflow of pus from the dilated cervix. Vaginal discharge in open cervix pyometra can be malodorous, sanguineous, or mucopurulent (Patil et al. 2013). In cases of closed cervix pyometra, there is no vaginal discharge, and this condition is associated with more severe general symptoms and abdominal enlargement (Jitpean et al. 2017). Haematological and biochemical changes usually occur in pyometra affected bitches (Shah et al. 2017, Maharathi et al. 2020). The preliminary diagnosis is based on history and findings on clinical examinations, hematology and blood biochemistry analyses, and ultrasonography (USG) and/or radiography of the abdomen (Hagman 2022). Ultrasonography provides the advantages of detecting intrauterine fluid, uterine size and uterine wall thickness (Uçmak et al. 2021). Common systemic signs of pyometra include vomiting, inappetance, polyuria/polydipsia and lethargy (Patil et al. 2013). Pyometra affects multiple organ systems, leading to systemic inflammatory response syndrome (Enginler et al. 2014). It is usually accompanied by glomerular and tubular dysfunction leading to renal failure (Ahuja 2019). Lippi et al. (2014), reported that detection of hyperphosphatemia and high serum calcium-phosphorus concentration should be considered possible predictors of negative outcome in dogs with chronic kidney disease. Asheim (1963), indicated that renal dysfunction in dogs occur in conjunction with potassium depletion and with hypercalcemia. Phosphorus may lead to fatty degeneration of a moderate degree in liver (Althausen and Thoenes 1932). Also, pyometra led to endotoxaemia which causes intrahepatic cholestasis and alteration of heptocellular function (Hagman et al. 2006). Decrease in albumin concentration may occur during the later stages of hepatic damage following hyperproteinemia (Uçmak et al. 2012).

The aim of this study to evaluate the changes in calcium, phosphorus and some biochemical parameters in dogs with open and closed cervix pyometra and to compare these with a control group.

Materials and Methods

Animals and study design

All animal procedures were carried out in accordance with the approval of the Unit Ethical Committee at the University of Istanbul- Cerrahpaşa Faculty of Veterinary Medicine (Approval number: 2022/45). A total of 62 bitches, aged 5-10 years and irrespective of breed, were enrolled in the study. All bitches were clinically (heart rate, respiratory rate, body temperature and dehydration status) and gynaecologically (vaginal cytology and transabdominal USG with 6.6 MHz convex transducer) examined. Twenty two healthy diestrus bitches were in Group H that presented to the clinic for ovariohysterectomy. On clinical examination, pyometra was diagnosed at 40 bitches (Group PYO) while 23 of 40 bitches had open-cervix pyometra (Group OCP) and 17 of 40 bitches had closed-cervix pyometra (Group CCP). The pyometra was treated with ovariohysterectomy. Before undergoing surgical intervention, hematological analyses were performed in all bitches.

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, Turkiye) according to the manufacturer's instructions. Slides were examined by using a light microscope at × 400 magnification. Totally, 300 cells were examined on each slide.

Gynaecological USG

Internal genital tract (ovaries and uterus) was examined trans-abdominally by the same operator in both groups with B-mode USG equipped with 6.6 MHz convex transducer (Esaote SPA. Genova, GE, Italy). The bitches were positioned and gently restrained. No sedation was used. The widest cross-sectional diameter of uterine body and presence of luminal content were visualized on left and right uterine horns by the USG equipment software (Uçmak et al. 2021).

Hematological analyses

The clinical examination, USG scanning and blood collection were performed at the same day. 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 hemogram (Procyte Dx Hematology Analyzer, Idexx, USA) were performed. White blood cell (WBC), glucose (Glu), creatinine

Table 1. The mean values and SEM of hematological parameters and their significances related to two groups.

Parameters (Reference ranges)	Group PYO (n=40)	Group H (n=22)	Significance
WBC (6-17 x10^9/L)	30.22±2.78[a]	12.13±0.56[b]	p<0.001
Glu (75-128 mg/dL)	93.88±2.40[a]	107.86±3.04[b]	p<0.001
Crea (0.4-1.4 mg/dL)	1.59±0.51	1.10±0.05	Ns
BUN (9.2-29.2 mg/dL)	20.82±5.90	14.32±1.37	Ns
BUN/Crea (12.5-31.8 mg/dL)	15.14±1.52	13.86±1.80	Ns
TP (5-7.2 g/dL)	7.63±0.11[a]	6.82±0.12[b]	p<0.001
Alb (2.6-4 g/dL)	2.82±0.07[a]	3.38±0.06[b]	p<0.001
Glob (2.5-4.5 g/dL)	4.82±0.13[a]	3.44±0.11[b]	p<0.001
Alb/Glob	0.61±0.03[a]	1.00±0.04[b]	p<0.001
ALT (17-78 U/L)	56.55±13.41	55.36±5.49	Ns
ALKP (13-83 U/L)	208.85±36.26[a]	65.50±13.11[b]	p<0.001
Ca (9.3-12.1 mg/dL)	9.68±0.12	9.48±0.12	Ns
Phos (2.9-5.3 mg/dL)	5.52±0.60	4.55±0.23	Ns

[a,b] 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

(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.

Statistical analysis

Statistical analyses were performed with SPSS 13.0 (SPSS Inc, Chicago, Illinois, USA). Normal distrubtion of the data was examined by Shapiro-Wilk test. Student's t-test and MannWhintney-U test were performed for the comparison of the Group PYO and Group H with regard to the hematological parameters. Differences between three groups (Group H, Group OCP, Group CCP) related to the evaluated parameters were determined by KruskalWallis test and Mann Whitney-U test. 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 of the bitches in group H and group PYO were 6±0.68 years and 8±0.45 years, respectively. Predominantly intermediate cells (20%), some parabasal cells (5%) and polymorphonuclear neutrophils (70%) were dense in cytology smears of all bitches in Group PYO. Also, large numbers of neutrophils (82% of the cytology slide) which were mostly degenerated, were detected in cases of open cervix pyometra. The background of the smear slides belonging to the bitches in Group OCP was not clean, showing evidence of pus and debris. The presence of pus was detected by microscopic examination with a wavy appearance on the surface of the slide. In bitches belonging to Group PYO, the cross-sectional uterine diameter was enlarged (mean ± SEM: 2.56 ± 0.28 cm) and uterine lumen was filled with pus. The mean uterine diameter of Group OCP was 2.08± 0.54 cm while it was 3.57±0.26 cm in Group CCP. The mean values and SEM of WBC, Ca, Phos and some biochemical parameters and their significances related to the Group PYO and Group H were presented in Table 1. Comparison of the evaluated parameters between the three groups (Group OCP, Group CCP, Group H) was given in Table 2. The relationships among the evaluated parameters were specified in Table 3.

Discussion

Pyometra is more common in dogs aged 6 years and older (Prasad et al. 2018). Alkan et al. (2020), determined the mean age of dogs with pyometra as 8.05±3.30 years, in their study. Similarly, in the present study, the mean age of the bitches in Group PYO was 8±0.45 years. Many studies revealed that dogs with pyometra have a significant rise in WBC value (Jitpean et al. 2017, Shah et al. 2017, Uçmak et al. 2021). In line with previous reports, the WBC count in Group PYO was found to be higher than in Group H (p<0.001) in this study. The increase in the WBC count is usually due

Table 2. The mean values and SEM of hematological parameters and their significances related to three groups.

Parameters	Group OCP (n=23)	Group CCP (n=17)	Group H (n=22)	Significance
WBC (10^9/L)	21.15±2.58[a]	42.50±3.95[b]	12.13±0.56[c]	p<0.001
Glu (mg/dL)	99.74±2.45[a]	85.94±3.89[b]	107.86±3.04[a]	p<0.001
Crea (mg/dL)	1.89±0.86	1.18±0.32	1.10±0.05	Ns
BUN (mg/dL)	20.40±8.93	21.38±7.16	14.32±1.37	Ns
BUN/Crea (mg/dL)	13.34±1.47	17.57±2.94	13.86±1.80	Ns
TP (g/dL)	7.65±0.15[a]	7.61±0.17[a]	6.82±0.12[b]	p<0.001
Alb (g/dL)	2.98±0.09[a]	2.60±0.10[b]	3.38±0.06[c]	p<0.001
Glob (g/dL)	4.66±0.20[a]	5.02±0.15[a]	3.44±0.11[b]	p<0.001
Alb/Glob	0.67±0.04[a]	0.53±0.02[b]	1.00±0.04[c]	p<0.001
ALT (U/L)	43.47±5.07	73.88±30.83	55.36±5.49	Ns
ALKP (U/L)	101.70±10.09[a]	353.82±79.81[b]	65.50±13.11[a]	p<0.001
Ca (mg/dL)	9.85±0.14[a]	9.44±0.20[b]	9.48±0.12[b]	p<0.05
Phos (mg/dL)	5.27±0.92[a]	5.91±0.68[b]	4.55±0.23[ab]	p=0.08

[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 3. The relationships among the evaluated parameters. Pearson correlation coefficents and significances.

	WBC	Glu	Crea	BUN	BUN/Crea	TP	Alb	Glob	Alb/Glob	ALT	ALKP	Ca	Phos
WBC	1	-0.595 ***	-0.068 ns	0.043 ns	0.326 *	0.291 *	-0.633 ***	0.550 ***	-0.620 ***	0.122 ns	0.377 **	-0.222 p=0.08	0.184 ns
Glu		1	0.110 ns	0.095 ns	0.046 ns	-0.276 *	0.561 ***	-0.491 ***	0.595 ***	0.085 ns	-0.388 **	0.258 *	0.002 ns
Crea			1	0.932 ***	-0.073 ns	-0.050 ns	0.177 ns	-0.131 ns	0.172 ns	-0.036 ns	-0.107 ns	0.038 ns	0.860 ***
BUN				1	0.147 ns	-0.099 ns	0.074 ns	-0.119 ns	0.121 ns	0.068 ns	-0.108 ns	-0.040 ns	0.915 ***
BUN/Crea					1	-0.084 Ns	-0.326 *	0.098 ns	-0.170 ns	0.465 ***	-0.054 ns	-0.321 *	0.199 ns
TP						1	-0.187 ns	0.871 ***	-0.607 ***	-0.036 ns	0.318 *	0.253 *	0.045 ns
Alb							1	-0.644 ***	0.855 ***	0.032 ns	-0.295 *	0.383 **	-0.019 ns
Glob								1	-0.897 ***	-0.049 ns	0.400 **	0.007 ns	0.041 ns
Alb/Glob									1	0.027 ns	-0.376 **	0.187 ns	-0.001 ns
ALT										1	-0.081 ns	-0.027 ns	0.078 ns
ALKP											1	-0.150 ns	-0.032 ns
Ca												1	-0.014 ns
Phos													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. * p<0.05 , ** p<0.01 , *** p<0.001, ns – p>0.05

to the number of immature neutrophils reaching up to 35%. The increase in the number of immature neutrophils is considered an indicator of the severity of the fever and the disease. In cases of pyometra, the increase in the number of leukocytes also causes a worsening of the prognosis (Prasad et al. 2018, Thangamani et al. 2018). Additionally, researchers have determined that the WBC count increases more in dogs with closed cervix pyometra and those in poor general condition (Jitpean et al. 2017, Alkan et al. 2020). Similarly, WBC level was higher in Group CCP than in Group OCP in this study ($p<0.001$). In dogs with closed cervix pyometra, due to the inability of the uterine content to come out, left-shift neutrophilic leukocytosis is more severe than in dogs with open cervix pyometra (Shah et al. 2017).

In many research, total protein concentration was found to be higher in dogs with pyometra than in healthy ones (Gupta et al. 2013, Enginler et al. 2014, Maharathi et al. 2020). However, Gupta et al. (2013), observed no difference in TP concentration between the dogs with close and open pyometra. Similar to the previous reports, TP level was significantly higher in Group PYO than in Group H ($p<0.001$) and no difference in TP levels was observed from the point of open or close pyometra in this study ($p>0.05$). The rise of TP in pyometra affected bitches may be attributed due to acute inflammatory changes and the dehydration status (Singh et al. 2006, Patil et al. 2013). Sepsis, chronic inflammation and endotoxemia cause protein loss by increasing vascular permeability (Patil et al. 2013). In cases of pyometra, hypoalbuminemia may occur due to decreased albumin synthesis and as there might be loss of albumin in urine (Singh et al. 2006). A decrease in albumin concentrations may occur during the later stages of hepatic changes following hyperproteinemia (Uçmak et al. 2012). The researchers (Fransson et al. 2004, Enginler et al. 2014) found significantly lower albumin concentrations in bitches with pyometra than in bitches with healthy uterus. Similar results were obtained in this study. The reason of this result may be because of the decreased production and increased loss of albumin in the presence of systemic inflammation (Werner and Turnwald 1999). Bacterial invasion stimulates the immune system and globulin production increases as a defense mechanism against the infection. Increase in globulin in bitches with pyometra could be due to an acute acute inflammatory changes and synthesis of antibodies in response to bacterial infection (Hagman 2006). Maharathi et al. (2020), observed significant increase in concentration of serum globulin in pyometra affected bitches. Similarly, hyperglobulinemia was observed in Group PYO. However, no distinct trend was observed in globulin levels of the bitches suffering from close and open pyometra in present study.

Another important problem seen in dogs with pyometra is changes in liver function. Hepatocellular damage, impaired hepatic circulation, intrahepatic cholestasis and cellular hypoxia as a result of endotoxemia are frequently encountered in dogs with pyometra (Prasad et al. 2018, Nak et al. 2001). The elevated ALKP levels were observed in many studies in dogs with pyometra (Gupta et al. 2013, Patil et al. 2013, Enginler et al. 2014). Similarly, ALKP level in Group PYO was significantly higher than in Group H in this study ($p<0.001$). Alkan et al. (2020), specifically determined that ALKP values increased more in dogs with closed-cervix pyometra than in dogs with open-cervix pyometra. Likewise, the highest ALKP level was observed in Group CCP of this study. An increase in ALKP level may be observed as a result of cholestasis and liver necrosis that occur as a result of endotoxemia (Sodikoff 1995).

Chung et al. (2003), suggested that low serum phosphorus levels in patients who recovered from hepatic failure may be associated with recovery of hepatic function. 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). Reduced level of glucose in Group PYO could be explained by the effect of increased Phos level to influence on carbohydrate metabolism as previously reported. A significant increase in serum Phos levels has been reported in dogs with chronic kidney disease (Lippi et al. 2014). Similarly, serum Phos level was significantly associated with the BUN and Crea levels ($p<0.001$; $R=0.913$ and $R=0.856$; respectively). Koo et al. (2011), reported the serum Phos levels within the reference ranges in two young dogs with closed--cervix pyometra. Günay Uçmak and Islamoglu (2023), determined the highest inorganic Phos level in cats with close cervix pyometra. Although the serum Phos level in this study was within the reference ranges, the Phos concentration in group CCP tended to increase compared to Group OCP ($p=0.08$). Particularly in cases of closed cervix pyometra, severe systemic clinical signs are often observed as renal and hepatic dysfunction (Kutzler et al. 2012). The hyperphosphatemia in Group CCP could be explained by negative impact of serum Phos levels on the hepatic and renal function (Chung et al. 2003, Lippi et al. 2014). Shin et al. (2014), detected that serum calcium and phosphorus levels are significantly associated with fatty liver disease in humans. Additionally, disruption of calcium and phosphorus homeostasis is frequently observed in humans with chronic kidney disease. (Block et al. 1998). However, Lippi et al. (2014), didn't observed differences in serum total calcium and ionized calcium concentration

between the dogs with chronic kidney disease and healthy ones. In contrast with Lippi et al. (2014), the serum Ca level in Group OCP was significantly higher than in both group CCP and Group H in our study ($p<0.05$). Also, serum Ca was positively correlated with glucose ($p<0.05$), total protein ($p<0.05$) and albumin levels ($p<0.01$). These results could be explained by the previous studies in humans that indicated the association of Ca and Phos with the hepatic and renal diseases (Block et al. 1998, Shin et al. 2015). To best of our knowledge, this was the first study to evaluate the serum Ca and Phos levels in regard to cervical patency in dogs with pyometra.

Since cases of closed cervix pyometra are relatively difficult to diagnose, determining typical blood parameters in these cases is clinically useful. In our study, we thought that presenting typical blood results in open and closed cervix pyometra cases would be very useful for clinicians. It was concluded that biochemical tests need to be well evaluted in order to reveal the multisystemic effects of pyometra in bitches. It would be useful to evaluate the bitches with pyometra by measuring the parameters indicating liver and kidney functions, as well as the amounts of elements such as calcium and phosphorus that play important role in the enzymatic processes in these organs.

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