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Haematobiochemical and Vaginal Cytological Studies in Canine Pyometra

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Abstract

In the present study an attempt was made to evaluate haematobiochemical and vaginal cytological changes in pyometra affected bitches (n=80) and was compared with control group (20). A total of 80 bitches of age group 2-13 years old irrespective of breed were clinically examined and diagnosed for pyometra. Haematological changes revealed that Hb, RBC, PCV and lymphocyte values were significantly ($P < 0.05$) low, whereas TLC & neutrophil values were significantly ($P < 0.05$) higher in pyometra affected bitches than control. Biochemical studies showed significant increase ($P < 0.05$) in the concentration of total protein (TP), globulin, SGOT and ALP, whereas albumin levels were significantly ($P < 0.05$) reduced. BUN and creatinine levels were significantly increased in pyometra affected bitches. Vaginal cytology in healthy bitches revealed predominance of parabasal and intermediate cells (>80%) with less/few superficial cells (<20%), reflecting diestrus stage of estrous cycle. Bitches with open pyometra exhibited more numbers of parabasal and intermediate cells and scanty superficial cells along with excessive numbers of degenerated neutrophils, intracellular and extracellular bacteria, whereas bitches with closed pyometra showed appearance of higher parabasal cells with few intermediate cells in vaginal cytology.

Keywords: Haematobiochemical changes, Vaginal cytology, Canine pyometra

Introduction:

Pyometra is a common illness in adult intact female dogs (Hagman, 2018). It is a hormonally mediated diestrual disorder. Pyometra usually occurs in older bitches over six years of age within twelve weeks of last estrus (Slatter, 1985). The clinical signs noticed by pet owners are vaginal discharge, depression, polyuria, polydipsia, abdominal distension, vomiting and anaemia (Liao et al., 2020). Several terms such as chronic endometritis, chronic purulent metritis or cystic endometrial hyperplasia complex, have been used in the literature to describe the condition (Fukuda, 2001). Progesterone has been shown to stimulate endometrial glandular secretion followed by suppressed contractions of the uterus, thus creating an intrauterine environment make susceptible to bacterial growth. Moreover, nulliparous bitches are highly prone for pyometra (Smith, 2006). Haematobiochemical changes that occur following pyometra have been proposed to be used as prognostic indicators for evaluation of pyometra affected bitches (Srinivas et al., 2018; Thangamani et al., 2018). Till now, studies of haematobiochemical changes along with the vaginal cytological changes in canine pyometra are not very common. Therefore, the aim of present study was to demonstrate haematobiochemical and vaginal cytological changes in pyometra affected bitches which are considered to be significant to evaluate the severity and type of pyometra.

Materials and Methods:

The present study was conducted on the clinical cases presented to the Veterinary Gynaecology and Obstetrics department of Veterinary Clinical Complex (V.C.C.), West Bengal University of Animal and Fishery Sciences, West Bengal, India during the period of August, 2023 to February, 2025. A total 80 pyometra affected bitches between 2 to 13 years of age were selected on the basis of case history and the clinical signs exhibited by them viz., distended abdomen, anorexia, vomiting, foul smelling sero-sanguineous to chocolate brown colour vaginal discharge, polyuria, polydipsia and uremic signs. Normal healthy animals (n=20) which came for sterilization were included in this study as control. Minimum 5ml of blood was collected in clot and EDTA vial from both i.e., pyometra affected bitches and control animals. From which, about 1ml of blood was poured into a sterile vial containing anticoagulant EDTA (2 mg/ml) and haematological studies was carried out within 3 to 4 hours from its collection using automated blood counter (Beckman Coulter, USA). Remaining blood was collected in a test tube and was allowed for clotting, serum was separated out by centrifugation and supernatant was then collected in sterile vials. The collected serum samples were analyzed for different biochemical parameters by

automated clinical chemistry analyzer (Vitros System Chemistry DT 60 11, Orthoclinical Diagnostics, Johnson and Johnson, USA).

Haematological parameters included were haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular value (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCHC), total leukocyte count (TLC), neutrophil, lymphocyte, monocyte, eosinophil, platelet and erythrocyte sedimentation rate (ESR). Whereas biochemical parameters studied were total protein (TP), albumin (A), globulin (G), A:G ratio, total bilirubin, serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), plasma glucose, blood urea nitrogen (BUN), creatinine and uric acid.

Vaginal cytology was performed to determine the types of epithelial cells of vagina and their responses in both, pyometra affected and normal healthy bitches as per protocol described by Bell et al. (1973).

Results and Discussion:

Haematological

The mean values of haemoglobin (g/dl), Red Blood Cell (RBC x 10^6/mm³), Packed cell Volume (PCV %), and Total leukocyte Count (TLC x 10³/UL) was recorded as 14.83± 0.28, 6.62 ± .09, 45.43 ± 0.80 and 9.87 ± 0.38, respectively in clinical group (Table 1). The corresponding mean values were 11.03 ± 0.34, 4.19 ± 0.11, 33.61 ± 0.97 and 26.04± 2.43, respectively in control animals. Significant ($p < 0.05$) reductions in hemoglobin (Hb), red blood cell count (RBC), and packed cell volume (PCV) were observed in pyometra-affected bitches but TLC values were increased. In pyometra affected bitches mean Erythrocyte Sedimentation Rate (mm/hr.) was slightly higher (19.80 ± 3.15). Haemoglobin, RBC and PCV values were decreased in bitches affected with pyometra indicating anemia which is in agreement with the findings of Jena et al. (2013) and Samantha et al. (2018). This might be due to loss of red blood cells by diapedesis into uterine lumen apart from depressed feed intake and impaired erythropoiesis under toxemic condition in severely affected cases as reported by Dabhi et al. (2009). PCV level was decreased in bitches affected with pyometra as compared to control animals, indicating a mild normocytic, normochromic and regenerative type of anaemia which is in agreement with the findings of Jena et al. (2013) which might be due to concomitant dehydration as reported by Verstegen et al. (2008). In the present study, leukocytosis and neutrophilia were the most consistent finding among the bitches affected with pyometra which was in agreement with the reports of Jena et al. (2013). Leukocytosis might be due to increased stress on the body defense mechanism which in turn produced increased leucocytes to combat the infection as reported by Nath et al. (2009), while neutrophilia might be due to influence of toxins in pyometra and their degenerative effect on the neutrophils as stated by Babu et al. (2017). Increased ESR in pyometra affected bitches might be due to the inflammatory response triggered by the infection, leading to higher levels of proteins like fibrinogen in the blood, which causes red blood cells to clump together and settle faster (Gupta and Dhami, 2013).

Biochemical

Total protein (TP) and globulin levels were significantly ($P<0.05$) elevated in diseased animals, whereas albumin levels were significantly ($P<0.05$) reduced in pyometra affected bitches, reflecting negative acute-phase protein response (Table 2). The albumin-to-globulin (A:G) ratio was lower in clinical cases, highlighting a strong immune and inflammatory response. The observed hyperproteinemia in this present study is in agreement with the finding of Gupta and Dhami, (2013).

Hyperproteinemia in these cases might be due to acute phase reaction in pyometra affected bitches (Singh et al., 2006). The Hyper-globulinemia, concurrent with hypoalbuminemia in this present study was in agreement with Reidun et al. (2007). Liver enzymes, Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Transaminase SGOT) and Alkaline Phosphatase (ALP) were elevated in dogs with pyometra which may be attributed to hepatic stress or systemic inflammation. Present findings are in accordance with Sachan et al. (2019) and Verstegen et al. (2008).

Blood urea nitrogen (BUN) and creatinine levels were significantly ($P<0.05$) increased in pyometra bitches, suggesting prerenal azotemia or early renal impairment due to septicemia. Elevated BUN and creatinine level might be due to toxaemia, in turn leading to decomposition of body protein as a result of suppurative process and reduced renal perfusion and dehydration leading to immune complex deposition in the glomeruli causing glomerulonephritis and proximal tubular damage resulting in renal failure depending upon the level of toxaemia and dehydration caused by pyometra as reported by Gayakawad et al. (1999) and Nath et al., (2009). However, Nak et al. (2004) reported that pyometra might cause renal failure due to the effect of bacterial toxins especially, *Escherichia coli* on renal tubules. In contrary, Hagman et al. (2009) reported that BUN and creatinine values of the affected bitches did not differ significantly from normal values demonstrating a normal kidney and liver function and lack of hepatocellular damage in the most dogs.

Vaginal cytology

Vaginal cytology in healthy bitches revealed that the vaginal epithelium exhibited more numbers of parabasal and intermediate cells (>80%) with few numbers of superficial cells (<20%), reflecting the di-estrous/diestrus stage of estrous cycle (Figure 1). Open pyometra affected bitches also showed excessive numbers of parabasal and intermediate cells with scanty superficial epithelial cells, degenerated neutrophils, intracellular and extracellular bacteria in protein background (Figure 2) while in bitches having closed pyometra, higher number of parabasal cells with few intermediate cells were found in cytological examination (Figure 3).

The present study was demonstrated excessive numbers of degenerated neutrophils, bacteria, parabasal cells and intermediate cells in vaginal cytology in bitches with open pyometra. In patients with closed pyometra the epithelial cells found are parabasal cells with decreased intermediate cells which were in accordance with Bosschere et al. (2001). Meanwhile, vaginal cytology findings in healthy bitches may only reflect the di-estrous/diestrus stage of estrous cycle.

Conclusion:

Significantly ($P<0.05$) lower values of hemoglobin (Hb), red blood cell count (RBC) and packed cell volume (PCV) were observed in pyometra-affected dogs compared to healthy controls. These reductions are indicative of anaemia, likely of inflammatory or chronic disease origin. Total leukocyte count (TLC) and neutrophil percentages were significantly ($P<0.05$) elevated in pyometra-affected bitches indicating a strong systemic inflammatory response. Total protein (TP) and globulin levels were significantly ($P<0.05$) elevated in diseased animals, whereas albumin levels were significantly ($P<0.05$) lower in affected bitches, reflecting negative acute-phase protein response. Renal function markers such as blood urea nitrogen (BUN) and creatinine were significantly ($P<0.05$) increased in pyometra, suggesting prerenal azotemia or early renal impairment due to septicemia. SGPT and SGOT were significantly elevated ($P<0.05$) in bitches with pyometra. Hardly any difference in the character of epithelial cells was found between the control and closed pyometra affected bitches and resembled diestrus condition. Whereas, in open pyometra cases, excessive degenerated neutrophils and bacteria were found.

Conflicts of Interest:

No conflict of interest among the authors.

Data availability:

Data will be available on request.

Authors' contributions:

All authors contributed for the study as and when they have required their need.

Ethical approval:

Not applicable.

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Table 1: Haematological parameters in pyometra affected bitches and control group

Parameters	Healthy animals (Control, n=20)	Pyometra affected bitches (Clinical, n=80)
Hb (gm/dl)	14.83 ± 0.28^{a}	11.03 ± 0.34^{b}
RBC (x 10^6/ µl)	$6.62 \pm .09^{a}$	4.19 ± 0.11^{b}
PCV (%)	45.43 ± 0.80^{a}	33.61 ± 0.97^{b}
MCV (/L)	68.56 ± 0.73	80.66 ± 1.22
MCH (pg)	22.37 ± 0.26	26.25 ± 0.34
MCHC (%)	32.64 ± 0.17	32.85 ± 0.31
TLC (x10^3/ µl)	9.87 ± 0.38^{a}	26.04 ± 2.43^{b}
Neutrophil (%)	66.40 ± 1.14^{a}	77.75 ± 1.27^{b}
Lymphocyte (%)	22.81 ± 0.88	14.53 ± 1.24
Monocyte (%)	4.65 ± 0.51	4.48 ± 0.45
Eosinophil (%)	5.55 ± 0.67	4.89 ±0 .29
Platelet (x10^3/ µl)	298.60 ± 16.63	179.38 ± 15.43
ESR (mm/hr)	8.00 ± .96	19.80 ± 3.15

NB: Means with different superscripts within a row differ significantly ($p < 0.05$) as per Duncan's multiple range test.

Table 2: Biochemical parameters in pyometra affected bitches and control group

Parameters	Healthy animals (Control, n=20)	Pyometra affected bitches (Clinical, n=80)
TP (gm/dL)	5.90 ± 0.10^{a}	6.50 ± 0.11^{b}
Albumin (gm/dL)	2.82 ± 0.08^{a}	$2.41\ 0.10^{b}$
Globulin (gm/dL)	3.09 ± 0.14^{a}	4.12 ± 0.13^{b}
A:G	0.95 ± 0.06	0.61 ± .039
Total Bilirubin (mg/dL)	0.16 ± .03	0.36 ± .02
SGPT (IU/L)	44.17 ± 3.13^{a}	77.36 ± 6.42^{b}

SGOT (IU/L)	52.54 ± 3.89	77.27 ± 6.19
ALP (IU/L)	59.82 ± 5.85[a]	179.11 ± 24.87[b]
Plasma Glucose (mg/dL)	75.55 ± 2.25	70.97 ± 1.48
BUN (mg/dL)	15.66 ± .86[a]	27.90 ± 3.55[b]
Creatinine (mg/dL)	0.830 ±.067[a]	1.49 ± .18[b]
Uric acid (mg/dL)	1.09 ± .10	0.99 ± 0.09

NB: Means with different superscripts within a row differ significantly ($p < 0.05$) as per Duncan's multiple range test.

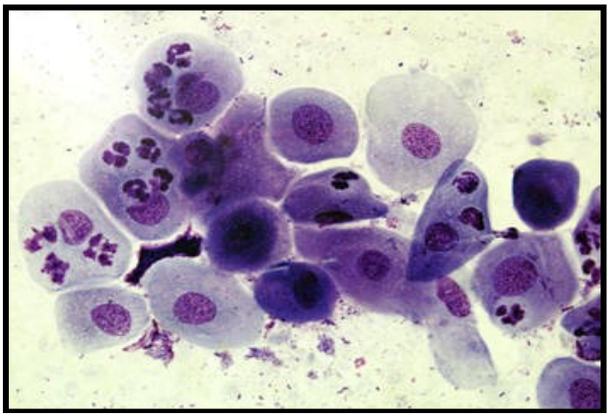

Figure 1: Vaginal cytology in a healthy bitch showing more numbers of intermediate and parabasal cells with less number of superficial cells reflecting diestrus stage of estrous cycle

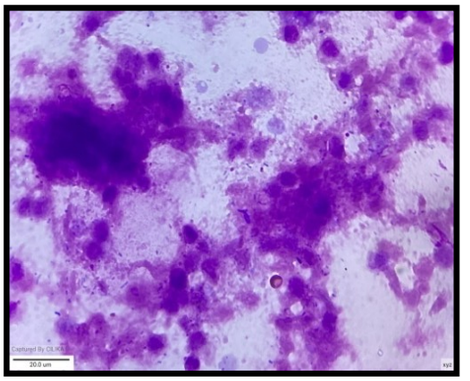

Figure 2: Vaginal cytology of a bitch with open pyometra showing degenerative neutrophils with intracellular and extracellular bacteria in protein background. Parabasal and intermediate cells were more in number whereas superficial cells are scant

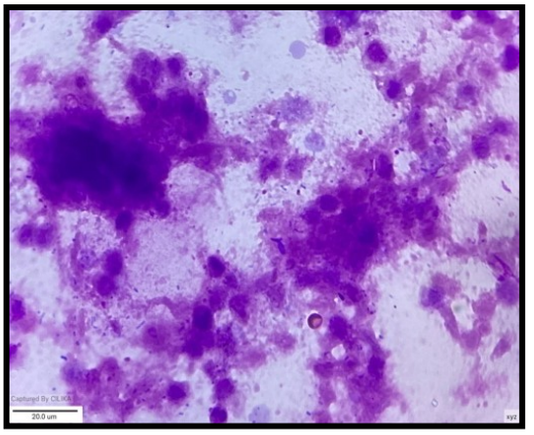

Figure 3: Vaginal cytology in a bitch with closed showing more numbers of parabasal cells with decreased numbers of intermediate cells

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