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Haemato-biochemical changes in pyometra affected bitches during and after ovariohysterectomy

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Abstract
The present investigation was carried out to study the haemato-biochemical changes in pyometra affected bitches during and after ovariohysterectomy in Group 1 (ovariohysterectomy by conventional method) and Group 2 (ovariohysterectomy by vessel sealant device). The results showed that, no significant changes in haemoglobin, packed cell volume and red blood cell count. Whereas, significant decrease in white blood cell count and significant increase in platelet count and significant decrease in alkaline phosphatase level was observed after ovariohysterectomy in both the groups during investigation periods. Further, method of ovariohysterectomy not influence the haemato-biochemical changes.

Keywords: Bitch, biochemical parameters, haematology, ovariohysterectomy, pyometra

Introduction
Pyometra is a common diestrual disease in adult bitches characterized by genital and systemic illness with a variety of clinical signs, pathological findings and inflammatory uterine accumulation (Kuplulu *et al*., 2009) [1] and is common in bitches over 5 years of age. Pyometra is known to culminate to systemic inflammation potentially affecting the multiple organ specifically kidney, liver and including heart (Pelander *et al*., 2008) [2]. The pathophysiological alteration of the uterus and haemato-biochemical changes that occur following pyometra have been proposed to be used as prognostic indicators for evaluation of pyometra affected bitches (Prasad *et al*., 2017; Srinivas *et al*., 2018 and Thangamani *et al*., 2018) [3-5].
Treatment consists of medical and surgical management. The advantages of surgical treatment (ovariohysterectomy) over the medical management are both curative and preventive (Fieni *et al*., 2014) [6]. Bipolar vessel sealing device is preferred when compared to other haemostatic technique like conventional suture ligation for open and laparoscopic ovariohysterectomy, since it reduces the cost indirectly by minimizing haemorrhage and reduction in surgical time (Janseen *et al*., 2012) [7]. Therefore the present study was undertaken to study the haemato-biochemical changes in pyometra affected bitches and evaluate the haemostatic technique in correlation with haemato-biochemical changes.

Materials and Methods
Location of the study: The study was carried out in the Department of Veterinary Surgery and Radiology, Small Animal Surgery Unit, Madras Veterinary College, Vepery, Chennai.

Selection of animals: The investigation included a total of 24 bitches (Group 1 (n=12), ovariohysterectomy by conventional method, whereas Group 2 (n=12) with vessel sealant device), which age from 5 years to 14 years and clinical signs are exhibited 5 to 8 days before presentation to the hospital. Tentatively diagnosed as pyometra based on the case history and peculiar clinical signs exhibited by the affected bitches which includes, distended abdomen, anorexia, vomition, sero-sanguineous to foul smelling chocolate brown colour pus discharge and uremic signs. All the bitches were subjected for ultrasonography or radiographic examination for confirmative diagnosis.

Blood collection: The haemato-biochemical parameters were recorded on the day of ovariohysterectomy (day 0) and 7th, 14th day after ovariohysterectomy. Blood was collected by cephalic veni-puncture using heparinized glass vials (10 IU heparin/ml blood) and clot activators.

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Quantification of haemato-biochemical parameters:
Haematological parameters includes, haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), Red blood cell count (RBC), White blood cell count (WBC) and platelet count were estimated.

Blood was centrifuged at 2500 rpm for 10 min and serum was separated and stored in labelled plastic vials at -20^0C until assay of biochemical parameters includes, blood urea nitrogen (BUN), Creatinine, Total protein, Albumin, Globulin, Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) were estimated.

Statistical analysis
All the parameters were recorded and analyzed statistically as per the standard procedure described by Snedecor and Cochran (1994) [8]. The parameters of the two different ovariohysterectomy methods were analysed by two sample t-test to understand the difference between groups on the specific day of treatment (Day 0, 7, 14) and one way ANOVA was performed to assess the difference between days within each ovariohysterectomy method. The significance of all the parameters were measured at *P*< 0.01 and *P*< 0.05 level significance.

Results and discussion
The haemato-biochemical parameters recorded in the pyometra affected bitches were tabulated in Table 1 to 3 and Figure 1 to 4.

Haematological alterations
The finding of the present study showed no significant difference in haemoglobin, packed cell volume and red blood cell count on 0, 7th and 14th day postoperatively in both the groups but the values were lesser than normal range indicating anemia. The white blood cell count on 0th postoperative day was above the normal range. A highly significant reduction in white blood cell count after 7 days postoperatively was recorded. A significant increase in platelet count was observed after 7 days postoperatively (Table 1 and Figure 1). This could be due to reversal of toxin present in the blood stream.

The present findings were in accordance with findings of Mojzisova *et al*. (2000), Singh *et al*. 2010 and Shah *et al*.

(2017) [9-11] who stated that leukocytosis with decreased lymphocyte count and anemia was predominant in closed cystic endometrial hyperplasia and pyometra complex (CEH-PC) due to toxemia. The present study suggested that method of ovariohysterectomy not influenced the haematological changes in pyometra affected bitches during post-operative period.
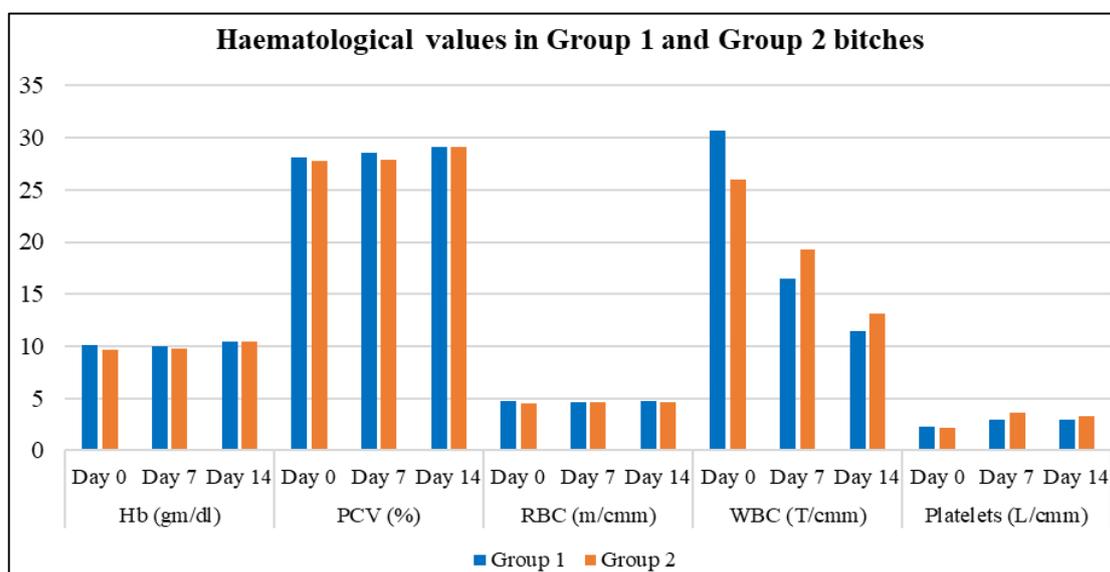
Differential leucocytes count
The findings of present study showed elevated level of neutrophils and decreased level of lymphocytes on the day of surgery. There was a significant decrease in the neutrophils counts and increases in lymphocytes count that was observed after 7 days postoperatively both the groups (Table 2 and Figure 2). This indicates the reversal of toxin in the blood. Statistically no significant difference was observed in monocytes counts.

Results of present findings were in consonance with the findings of Kaymaz *et al*. (1999), Mojzisova *et al*. (2000), Singh *et al*. 2010 and Shah *et al*. (2017) [9-12] who stated that 73.3 per cent of the dogs with pyometra had higher level of WBC counts. The present study inferred that method of ovariohysterectomy not influenced the differential leucocytes count in pyometra affected bitches during post-operative period.

Biochemical alterations
Kidney and liver functions were evaluated by serum levels of BUN, creatinine, total protein, albumin, AST and ALP (Figure 3 and 4). The present findings showed that there was no significant difference in BUN, creatinine, total protein, albumin and ALT concentrations on 0, 7th and 14th days postoperatively but ALP was higher than normal range and there was a significant decrease in ALP level observed after 7th post-operatively day in both the groups (Table 3).

In the present study, presence of hepatic injury due to toxemia as opined by Colombo *et al*. (1988), Verstegen *et al*. (2008) and Shah *et al*. (2017) [11-14] during diagnosis of condition and reversal of hepatic injury during post-treatment period. The present study inferred that method of ovariohysterectomy not influenced the biochemical parameters in pyometra affected bitches during post-operative period.
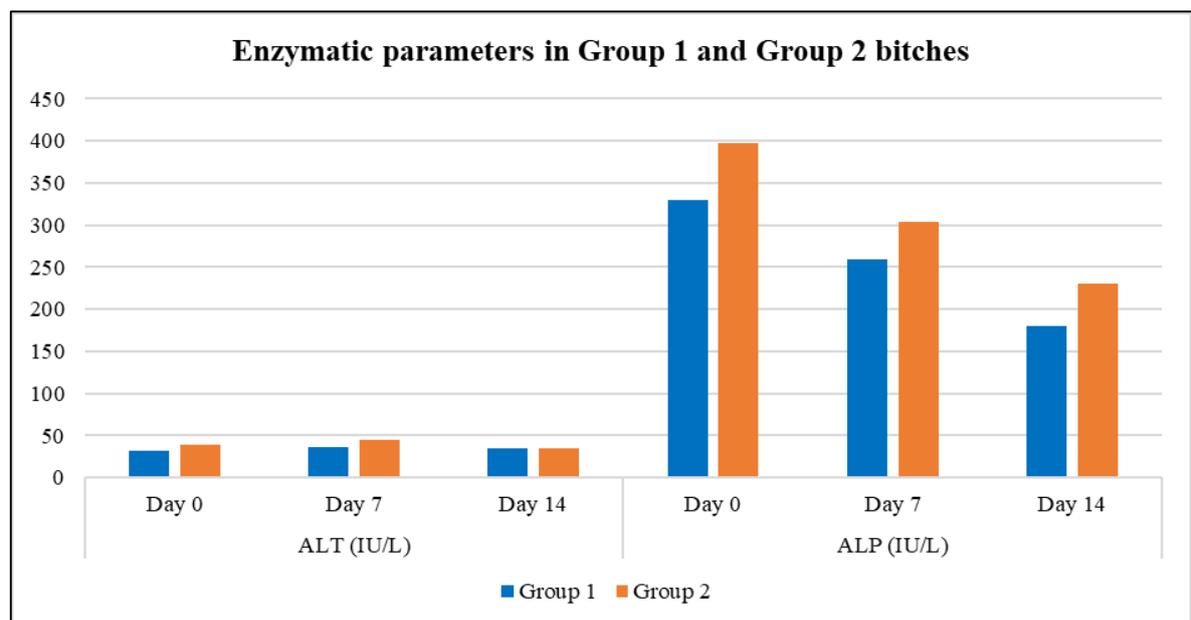

Fig 1: Haematological values in Group 1 and Group 2 pyometra affected bitches
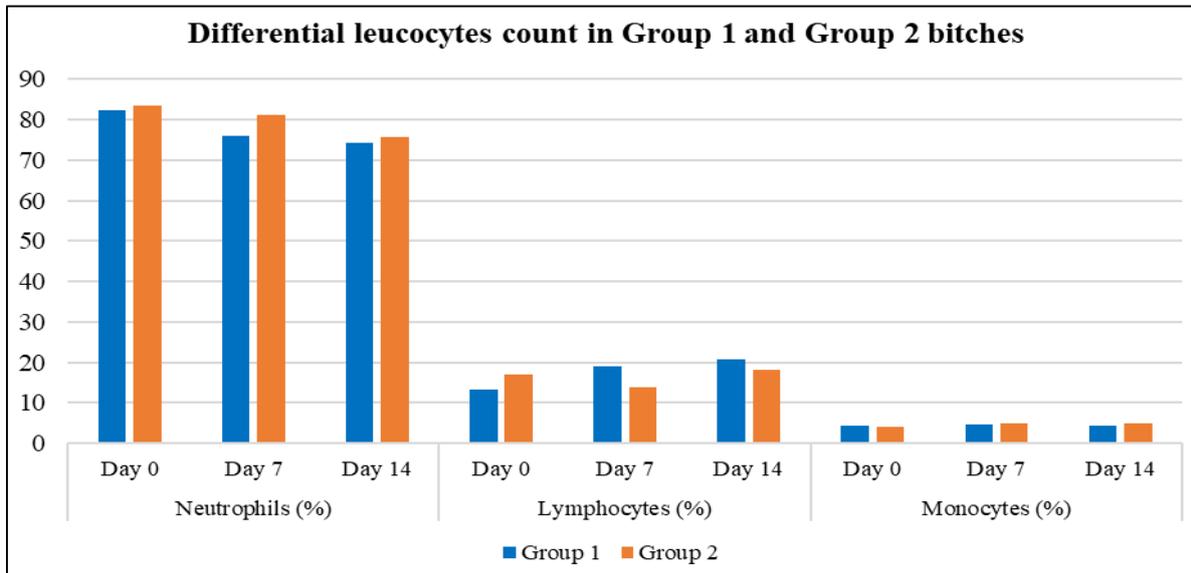
Fig 2: Differential leucocytes count in Group 1 and Group 2 pyometra affected bitches
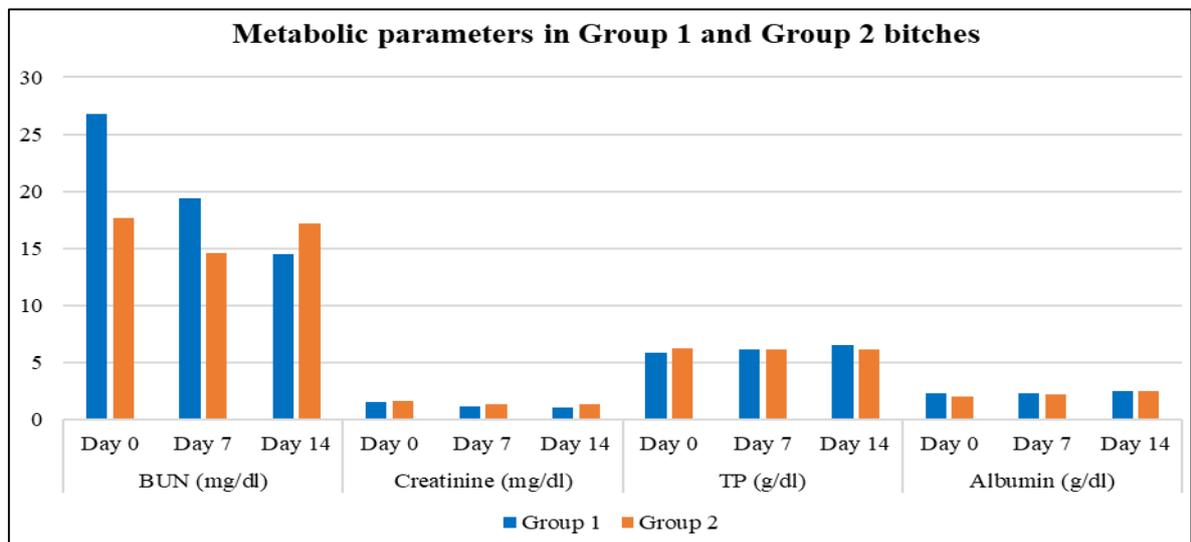
Fig 3: Metabolic parameters in Group 1 and Group 2 pyometra affected bitches

Fig 4: Enzymatic parameters in Group 1 and Group 2 pyometra affected bitches

Table 1: Mean±SE of haematological values on 0, 7th and 14th post-operative days

Parameter	Group/ Days	Mean±SE				F-value
		0	**7**	**14**	**Total**	
Hb (g/dl)	Group-I	10.127^a ±1.02	9.973^a±0.82	10.473^a±0.87	10.191±0.51	0.080^{NS}
	Group-II	9.683^a±0.75	9.808^a±0.63	10.392^a±0.71	9.961±0.39	0.292^{NS}
	t-value	0.354^{NS}	0.160^{NS}	0.073^{NS}		
PCV (%)	Group-I	28.091^a±2.71	28.600^a±2.32	29.145^a±2.00	28.612±1.32	0.050^{NS}
	Group-II	27.775^a±2.43	27.883^a±1.67	29.142^a±1.55	28.267±1.08	0.156^{NS}
	t-value	0.087^{NS}	0.253^{NS}	0.002^{NS}		
RBC (m/cmm)	Group-I	4.796^a±0.45	4.596^a±0.33824	4.7373^a±0.34	4.710±0.21	0.074^{NS}
	Group-II	4.542^a±0.33	4.662^a±0.27	4.640^a±0.24	4.615±0.16	0.053^{NS}
	t-value	0.466^{NS}	-0.155^{NS}	0.238^{NS}		
WBC (Thousands /cmm)	Group-I	30645.45^b±5237.42	16463.64^a±3428.66	11472.73^a±2518.19	19527.27±2608.38	6.519**
	Group-II	25941.67^b±5036.24	19283.33^{ab}±2678.22	13100.00^a±1540.96	19441.67±2107.84	3.544*
	t-value	0.647^{NS}	-0.654^{NS}	-0.562^{NS}		
PLATELET (lakhs /cmm)	Group-I	223081.82^a±39229.34	293636.36^a±46707.07	300745.45^a±41718.57	272487.88±24642.45	1.013^{NS}
	Group-II	216250.00^a±21778.54	363916.67^b±49164.14	329758.33^{ab}±50235.03	303308.33±26098.14	3.311*
	t-value	0.156^{NS}	-0.032^{NS}	-0.440^{NS}		

Means bearing different superscripts in a row differ significantly *($P< 0.05$), **($P< 0.01$) and NS- Non-significant ($P> 0.05$)

Table 2: Mean±SE of Differential Count of WBC's of on 0, 7th and 14th post-operative days

Parameter	Group	Mean±SE				F-value
		0th day	**7th day**	**14th day**	**Total**	
Neutrophils	Group-I	82.27 ^{b}±2.00	76.09^a ±1.83	74.36 ^{a}±1.94	77.58 ±1.232	4.671*
	Group-II	83.50 ^{b}±2.31	81.33^{ab} ±2.40	75.67 ^{a}±1.62	80.17 ±1.32	3.583*
	t-value	-0.398^{NS}	-1.714 ^{NS}	-0.519^{NS}		
Lymphocytes	Group-I	13.36^a±1.78	19.00^b±1.59	20.73^b±1.92	17.70±1.13	4.729*
	Group-II	16.92^a±5.32	13.75^a±2.30	18.25^a±1.41	16.31±1.946	0.456^{NS}
	t-value	-0.611^{NS}	1.896^{NS}	1.050^{NS}		
Monocytes	Group-I	4.36^a±0.24	4.64^a±0.39	4.36^a±0.39	4.45±0.195	0.206^{NS}
	Group-II	4.00^a±0.48	4.83^a±0.30	5.00^a±0.37	4.61±0.23	1.905^{NS}
	t-value	0.660^{NS}	-0.407^{NS}	-1.189 ^{NS}		

Means bearing different superscripts in a row differ significantly *($P< 0.05$), **($P< 0.01$) and NS-Non-significant ($P>0.05$)

Table 3: Biochemical values (Mean±SE) on 0, 7th and 14th day post-operative days

Parameters	Group	Mean±SE				F-value
		0th day	**7th day**	**14th day**	**Total**	
BUN (mg/dl)	Group-I	26.819^a±8.99	19.348^a±4.76	14.533^a±3.03	20.233±3.54	1.020^{NS}
	Group-II	17.691^a±2.18	14.642^a±1.93	17.173^a±3.23	16.502±1.42	0.422^{NS}
	t-value	1.027^{NS}	0.946^{NS}	-0.594^{NS}		
CREATININE (mg/dl)	Group-I	1.524^a±0.30	1.173^a±0.20	1.110^a±0.13	1.269±0.13	0.978^{NS}
	Group-II	1.658^a±0.17	1.395^a±0.13	1.334^a±0.25	1.462^a±0.11	0.824^{NS}
	t-value	-0.392^{NS}	-0.932^{NS}	-0.780^{NS}		
TOTAL PROTEIN (g/dl)	Group-I	5.909^a±0.37	6.136^a±0.30	6.564^a±0.25	6.203±0.18	1.133^{NS}
	Group-II	6.258^a±0.32	6.200^a±0.23	6.158^a±0.25	6.206±0.15	0.035^{NS}
	t-value	-0.714^{NS}	-0.170^{NS}	1.138^{NS}		
ALBUMIN (g/dl)	Group-I	2.300^a±0.11	2.330^a±0.15676	2.515^a±0.09	2.382±0.07	0.938^{NS}
	Group-II	2.055^a±0.13	2.240^{ab}±0.14	2.483^b±0.13	2.259±0.08	2.543^{NS}
	t-value	1.404^{NS}	0.433^{NS}	0.200^{NS}		
ALT (IU/L)	Group-I	32.27^a±3.37	36.55^a±2.82	35.27^a±1.97	34.70±1.59	0.622^{NS}
	Group-II	39.08^a±2.64	44.25^a±3.68	34.33^a±4.24	39.22±2.12	1.916^{NS}
	t-value	-0.605^{NS}	-1.639^{NS}	0.195^{NS}		
ALP (IU/L)	Group-I	329.36^b±32.10	259.73^{ab}±31.06	180.55^a±17.68	256.55±18.86	7.208**
	Group-II	397.42^b±52.44	304.00^{ab}±37.21	230.17^a±24.77	310.53±25.12	4.438*
	t-value	-1.083^{NS}	-0.904^{NS}	-0.913^{NS}		

Means bearing different superscript in a row differed significantly *($P< 0.05$), **($P< 0.01$) and NS-Non-significant

Conclusion

Evaluation of haemato-biochemical parameters facilitate the prediction of prognosis of pyometra affected bitches. The present study, concluded that there was early presentation of case with institution of intravenous fluid therapy, antibiotic cover and postoperative management based on haemato-biochemical parameters improves the survivability of the pyometra affected bitches. Further, method of ovariohysterectomy not influenced the haemato-biochemical changes in pyometra affected bitches during post-operative period.

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