

EVALUATION OF CLINICAL BIOCHEMISTRY OF BLOOD IN BITCHES AFFECTED WITH PYOMETRA*

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ABSTRACT

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Nine pyometric and eight healthy bitches were included to evaluate their plasma biochemical, enzymatic and sex steroid profile before and after ovario-hysterectomy. The pre-operative levels of plasma urea nitrogen (PUN), creatinine and total cholesterol were significantly ($P < 0.01$) higher in pyometric bitches than the normal ones (60.78 ± 10.03 vs 16.75 ± 1.58 mg/dl; 2.71 ± 0.51 vs 0.85 ± 0.10 mg/dl and 256.52 ± 25.19 vs 189.00 ± 8.91 mg/dl, respectively). The post-operative levels of all three constituents, particularly PUN and creatinine, declined in cases of pyometra. Plasma total protein (7.67 ± 0.61 vs 5.70 ± 0.28 g/dl) and globulin (5.29 ± 0.41 vs 2.30 ± 0.13 g/dl) concentrations were significantly ($P < 0.01$) higher and albumin (2.38 ± 0.22 vs 3.40 ± 0.28 g/dl) concentration was lower in pyometric bitches than the healthy ones, thus significantly ($P < 0.01$) reducing the albumin-globulin ratio (0.45 ± 0.02 vs 1.52 ± 0.15) in pyometra affected bitches. The pre-operative mean aspartate and alanine aminotransferase (AST, ALT) and alkaline phosphatase (ALP) activities were significantly higher ($P < 0.01$) in bitches affected with pyometra (66.33 ± 9.84 and 73.78 ± 8.78 IU/l, and 86.78 ± 13.20 KAU %, respectively) as compared to healthy ones (32.50 ± 1.70 , 31.25 ± 1.46 IU/l and 40.13 ± 2.59 KAU %). The levels of ALP were insignificantly higher in cases of closed pyometra than the open ones, but not of AST or ALT. The levels of all 3 enzymes dropped a little by 8-15 days following surgery in affected animals. Plasma progesterone profile was three times elevated (11.20 ± 2.88 vs 4.10 ± 0.91 ng/ml) and oestradiol-17 β was insignificantly reduced (623.78 ± 235.75 vs 777.38 ± 174.02 pg/ml) in pyometric bitches than the normal ones. Moreover, the mean progesterone concentration was significantly higher in cases of closed pyometra than the open ones (19.97 ± 2.88 vs 6.81 ± 2.59 ng/ml), and it declined significantly within 8-15 days following ovario-hysterectomy. It was concluded that significant elevation in serum biochemical and enzymatic profile together with progesterone in pyometric bitches as compared to normal ones with the drop in their levels post-operatively is of diagnostic and prognostic value in canine pyometra.

Key words: Bitch, Pyometra, Clinical Biochemistry, Ovario-hysterectomy.

INTRODUCTION

Pyometra is a common metoestrous disease of intact bitches with systemic illness (Borresen and Skrede, 1980; Sevelius *et al.*, 1990). An exaggerated response of the uterine mucosa to chronic progestational stimulation during the luteal phase due to altered

oestrogen-progesterone receptors leads to cystic endometrial hyperplasia with excess secretions in the uterine lumen and secondary bacterial infection particularly of *E. coli* from vagina that liberate endotoxin leading to organ damage, altered haemato-biochemical and enzymatic profile, and variety of clinical symptoms (Gayakwad *et al.*, 1999; Hagman, 2004). Significant increase in serum transaminases (Ettinger and Feldman, 1993; Hagman, 2004) and phosphatase (Sevelius *et al.*, 1990; Hagman, 2004) together with altered haemato-biochemical indices, particularly leucocytosis and elevated BUN and creatinine

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(Hagman, 2004; Dabhi and Dhama, 2006) have been reported earlier in bitches affected with pyometra. This study was focused on evaluating the plasma biochemical, enzymatic and sex steroidal profile of healthy and pyometric bitches before and after ovario-hysterectomy.

MATERIALS AND METHODS

Seventeen bitches presented for treatment of pyometra (n=9) or spaying (n=8) at Veterinary Teaching Clinical Services Complex of the College at Anand during the year 2011-12 were included in this study. Following retrieval of detailed history of each patient from the owner and its clinical and radiological examinations, ovario-hysterectomy was performed as per the standard technique through a mid-line approach as a treatment of pyometra or for elective sterilization. From all the bitches, whole blood samples were collected from cephalic vein in vials containing EDTA twice, i.e. just before operation and 15 days after the ovario-hysterectomy. The plasma was separated by centrifugation of blood samples at 3000 rpm for 15 minutes, and stored at -20°C until analyzed.

The levels of plasma urea nitrogen (PUN), creatinine, total cholesterol, total protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined by using standard procedures and assay kits of Crest Biosystem, Goa, India on an autoanalyzer. The levels of plasma globulin were derived by subtracting albumin from the total proteins and A : G ratios were worked out for all the samples. The plasma progesterone and oestradiol levels were estimated by employing standard RIA techniques. Labelled antigen (with I^{125}), antibody coated tubes and standards were procured from Immunotech, Marseille, Cedex, France. The sensitivity of the progesterone and oestradiol-17 β assays was 30 and 4 pg/ml. Their intra-assay variations were 5.4 and 15.1 %, and inter-assay variations 9.1 and 14.4 %, respectively.

Data obtained from individual bitches with open pyometra (6), closed pyometra (3) and normal spaying

(8) pre- and post-operatively were analyzed statistically using completely randomized design and 't' test.

RESULTS AND DISCUSSION

The pre-operative mean plasma urea nitrogen and creatinine concentrations were significantly higher ($P < 0.01$) in pyometric bitches $\{60.78 \pm 10.03$ (range 26.23 – 112.23) and 2.71 ± 0.51 (range 0.80 – 4.70) mg/dl, respectively} than in healthy bitches $\{16.75 \pm 1.58$ (9.00-29.00) and 0.85 ± 0.10 (0.40-1.20) mg/dl}. The levels were also higher in cases of open pyometra than the close ones, but did not differ significantly. These findings on plasma urea nitrogen compared well with the reports of Gayakwad *et al.* (1999) and Nak *et al.* (2004) and those of creatinine with the reports of Stone *et al.* (1988), Wakankar (1993) and Dabhi and Dhama (2006).

In present study, the plasma urea nitrogen level > 35 mg/dl was recorded in 77.78 % pyometric bitches and creatinine > 1.5 mg/dl in 66.67 % bitches. This compared well with the observations of Sharma (2004). The plasma concentrations of both urea nitrogen and creatinine decreased significantly by 8-15 days following surgery as compared to pre-operative values in pyometric bitches (50.02 vs 60.78 and 1.92 vs 2.71 mg/dl, respectively). This decline suggested reversal of toxemia and supported the earlier views of Borresen (1980) and Gayakwad *et al.* (1999). Urea nitrogen and creatinine both are catabolic waste products normally excreted by the kidneys and therefore both are indicators of glomerular filtration rate with which they are negatively correlated (Neel and Grindem, 2000). These could be useful guide to the prognosis of pyometra, irrespective of its type (De Coster *et al.*, 1979), since degree of damage to kidneys depends upon the level of toxemia and dehydration caused by pyometra. The post-operative decline in their levels was thus better prognostic for survival of the affected animals.

The plasma total cholesterol concentration was significantly ($P < 0.05$) greater in pyometric bitches than in healthy ones (256.52 ± 25.19 vs 189.00 ± 8.91 mg/dl), and it was higher in close pyometra than open

pyometra (293.92 ± 58.45 vs 237.82 ± 25.09 mg/dl). Bitches with close pyometra had multiple cysts and CLs simultaneously on their ovaries as visualized upon ovario-hysterectomy. Moreover, the cholesterol levels declined significantly ($P < 0.01$) in pyometric bitches within 8-15 days following ovario-hysterectomy. This might be due to change in hepatic dynamics and altered metabolism post-operatively. These observations closely corroborated with the reports of Borresen and Skrede (1980), Hagman (2004), Dabhi and Dhimi (2006) and Hagman *et al.* (2009). The elevated plasma concentrations of cholesterol probably reflect intrahepatic cholestasis in pyometric bitches (Sevelius *et al.*, 1990; Hagman (2004).

The pre-operative mean plasma total protein concentrations was significantly ($P < 0.05$) higher in pyometric bitches than the healthy bitches (7.67 ± 0.61 (range 5.70 – 10.34) vs 5.70 ± 0.28 (range 4.10 – 6.60) g/dl), but there was no difference between close and open pyometra, though the levels declined significantly within 8-15 days following surgery in pyometric cases. This finding of hyperproteinaemia observed in pyometric bitches coincided well with the previous reports of Dabhi and Dhimi (2006) and Hagman *et al.* (2009). The increased BUN and total plasma protein values in pyometric bitches suggested renal dysfunction probably due to pre-renal uremia, dehydration and toxemia (Jones and Joshua, 1988). Hardy and Osborne (1974) stated that the *E. coli* organisms release endotoxin, which interferes with the reabsorption of sodium and chloride in the loop of Henle impairing collecting tubules' ability to reabsorb the water, hence renal dysfunction occurs leading to polyuria and polydipsia in pyometric bitches. The hyperproteinaemia in pyometric bitches could be due to elevated globulin component.

In present study, the mean plasma albumin concentration of pyometric bitches was significantly ($P < 0.01$) lower (2.38 ± 0.22 vs 3.40 ± 0.28 g/dl) and globulin higher (5.29 ± 0.41 vs 2.30 ± 0.13 g/dl) than in healthy bitches, thus significantly ($P < 0.01$) reducing the albumin-globulin ratio (0.45 ± 0.02 vs 1.52 ± 0.15) in pyometra affected bitches. The observations of hypoalbuminaemia and hyper-globulinaemia recorded

in pyometric bitches corroborated well with the reports of Hagman (2004) and Dabhi and Dhimi (2006). These alterations occur due to loss of albumin via the damaged kidneys and increased production of α -globulin as a defense mechanism against infection (Borresen, 1980), since bacterial invasion stimulates the immune system to form immune complex at the basement membrane of glomeruli and it affects the glomerular filtration rate, thus causing renal dysfunction in pyometric bitches (Stone *et al.*, 1988). The present findings of marked increase in globulin over albumin fraction in pyometric bitches could be due to an acute phase reaction and synthesis of antibodies in response to bacterial infection as mentioned by Borresen and Skrede (1980) and Hagman (2004). However, in the present study no distinct trend was observed in the protein profile of bitches suffering from close and open pyometra, even before or after ovario-hysterectomy.

The mean activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were significantly ($P < 0.01$) higher in bitches affected with pyometra (66.89 ± 7.05 (range 24 – 89) and 73.78 ± 8.78 (range 55 – 112) IU/l, respectively), as compared with healthy bitches (32.50 ± 1.70 (27 – 35) and 31.25 ± 1.46 (25 – 37) IU/l), however, there was no difference in their profile between close and open pyometra. The AST level dropped significantly, but little drop was observed in ALT level following surgery only in affected animals. These findings of elevated plasma AST and ALT corroborated well with the reports of De Schepper *et al.* (1987) and Ettinger and Feldman (1993), respectively. Hagman (2004), however, stated that serum ALT values were within normal range even in pyometric bitches, while De Schepper *et al.* (1987) and Dabhi *et al.* (2007) observed increased levels of serum AST due to inhibition of liver enzyme synthesis or hepatic membrane damage.

The pre-operative mean plasma alkaline phosphatase (ALP) activity was also markedly elevated ($P < 0.01$) in pyometric bitches than the normal ones (86.78 ± 13.20 (range 46-155) vs 40.13 ± 2.59 (range 29-51) KAU/100 ml). The levels were also non-significantly higher in cases of close pyometra than

the open ones (107.00 ± 25.51 vs 76.67 ± 15.05 KAU%). There was significant ($P < 0.05$) decline in the plasma ALP activity within 8-15 days following ovario-hysterectomy in pyometric bitches. Sevelius *et al.* (1990), Ettinger and Feldman (1993) and Dabhi *et al.* (2007) reported similar findings in pyometric and normal bitches. Hagman (2004) and Hagman *et al.* (2009) found almost 3-4 times increase in plasma ALP activity in pyometric bitches as compared to healthy ones, which was however double in the present study. These findings reflect damage to the vital organs and bone marrow due to toxemia in pyometra of bitches.

The pre-operative plasma progesterone concentration recorded in bitches affected with pyometra (11.20 ± 2.88 (0.78 to 25.5) ng/ml) was nearly three times elevated ($P < 0.05$) than in normal ones (4.10 ± 0.91 (0.20 to 8.40) ng/ml). It was also significantly higher in close pyometra than the open ones (19.97 ± 2.88 vs 6.81 ± 2.59 ng/ml). This was associated with presence of multiple cysts and 1-3 CLs simultaneously on the ovaries in bitches with close pyometra and only 1-2 CLs in open pyometra. Its levels also declined significantly ($P < 0.05$) within 8-15 days following ovario-hysterectomy in all the animals due to removal of ovaries/CLs, since CL is the primary source of progesterone in circulation. The plasma oestradiol-17b concentration in pyometric bitches in contrast was apparently lower (623.78 ± 235.75 (43 to 1826 pg/ml) than in healthy bitches (777.38 ± 174.02 (26 to 1548) pg/ml). It was also slightly higher in close pyometra than the open ones.

The present findings of progesterone levels closely coincided with the reported value by Dabhi *et al.* (2007), but were slightly higher than those of Wankankar (1993), Karmakar *et al.* (2002) and lower than those of Nomura *et al.* (1984), especially in cases of close pyometra. Gao *et al.* (2011) also reported elevated plasma progesterone profile in pyometric bitches. The levels, in general, were not always higher in pyometric bitches under study than in normal ones in luteal phase as has been observed by others (Nomura *et al.*, 1984; Wakankar, 1993). The findings of oestradiol corroborated with the reports of Dabhi *et al.* (2007) and Gao *et al.*

(2011). Hardy and Osborne (1974) suggested that pyometra was the result of excessive and/or prolonged stimulation of uterus by the progesterone from the retained or cystic CL. Oestradiol concentration showed significant rise in cases of close pyometra, but not in healthy and open pyometra cases, following ovario-hysterectomy, probably due to development of new follicles on retained ovarian tissue.

Thus from the present findings, it was concluded that significantly elevated profile of plasma urea nitrogen, creatinine, transaminases and alkaline phosphatase as well as cholesterol, proteins and progesterone together with hypoalbuminaemia and hyperglobulinaemia observed in pyometric bitches as compared to healthy ones were of diagnostic and prognostic value, and indicated vital organ damage induced by bacterial endotoxins leading to increased production of α -globulin as a defense mechanism, and lack of production of uterine luteolysin from the damaged uterus that predisposed the animal to develop pyometra. Normalization of above profile following ovario-hysterectomy suggested reversal of toxemia and threat to the life of affected bitches.

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