



Original Research

A Comparative Study on Ultrasonographic and Bio-Chemical Changes with Histopathological Changes of Uterus in Pyometra Affected Bitches

S. Manokaran^{1*}, R. Ezakial Napolean², M. Selvaraju³ and G. A. Balasubramaniam⁴

Veterinary College and Research Institute, Namakkal – 637001, Tamil Nadu, INDIA

¹Kangayam Cattle Research Station, Sathyamangalam, Erode – 638402, Tamil Nadu, INDIA

²Department of Clinics

³Department of Veterinary Gynaecology and Obstetrics

⁴Department of Veterinary Pathology

*Corresponding author: smanokaran@rediffmail.com

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Abstract

The aim of the study was to compare the bio-chemical and histopathological changes with the ultrasonographic findings of uterine wall thickness in bitches affected with pyometra. The study was conducted in 30 experimental and 10 control bitches. There was a significant ($P < 0.05$) elevation in the serum BUN, creatinine, globulin and total protein levels in pyometra affected bitches. During ultrasonographic examination the uterine wall thickness was measured using electronic calipers. In normal bitches the uterine wall thickness was 2.56 mm whereas the thickness increased from 8.92 mm to 18.23 mm in the affected bitches. The biochemical profiles were correspondingly higher as the uterine wall thickness increased. The loss of endometrial surface, enlargement of endometrial glands and bursting of glands were noticed during histopathological examination as the wall thickness increased.

Key words: Bitches, Biochemical, Histopathology, Pyometra, Ultrasonography

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Introduction

Excessive or prolonged oestrogenic or progestogenic stimulation, whether natural or exogenous, induces endometrial changes in the bitch (De Cock *et al.*, 1997 and Sneha *et al.*, 2017). Cystic endometrial hyperplasia (CEH) is considered to be an exaggerated response of the uterus to chronic progestational stimulation during the luteal phase of the oestrous cycle, causing an abnormal accumulation of fluid within the endometrial glands and uterine lumen (Mustafa *et al.*, 1999). The resulting lesions of pyometra are due





to the interaction between bacteria and hormones. There are different therapeutic protocols used to treat pyometra starting from medical management to ovario-hysterectomy (Jena *et al.*, 2013). The treatment depends on the severity and duration of the disease, presence of pyometra alone or pyometra with CEH complex and the extent of damage to uterus and kidney. During ultrasonographic examination, the accumulated fluid inside the uterus could be visualized as anechoic area in bitches affected with pyometra. In cystic endometrial hyperplasia-pyometra complex the changes will be severe which could be reflected in the thickness of uterine wall along with the presence of exudates. The details or changes in the endometrium and deep uterine layers could not be studied with ultrasonography but could be best visualized with histopathological studies (Bigliardi *et al.*, 2004). With the above points in view, the present study was conducted to correlate the changes in the blood biochemical profile and histopathology of uterus to assess the involvement and damage to the uterine endometrium.

Materials and Methods

Thirty bitches presented to Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal with the history of lethargy, anorexia, vomiting, polyurea, polydypsia and purulent vaginal discharge were confirmed to have pyometra or cystic endometrial hyperplasia-pyometra complex by clinical and ultrasonographic examination and were then used as experimental animals. The clinically normal non-pregnant bitches (n=10) brought for ovario-hysterectomy served as control animals.

The ultrasound examination was performed by Caris (Esaote Biomedica, Florence, Italy) ultrasound scanner using 4-5-7 MHz sectoral probes. During trans-abdominal uterine ultrasonography, nature and extent of pyometra was studied. The uterus was examined to evaluate the integrity of endometrium, presence of exudates and cystic hyperplasia of endometrial glands. The thickness of uterine wall was measured by inbuilt electronic calipers in both control and experimental bitches. The ovario-hysterectomy was performed as per standard technique through midline approach as a treatment of pyometra. Tissue samples (1 cm²) were collected from the middle portion of the uterine horn and stored in 10 per cent formalin. 5 mm microtome sections were made and stained with haematoxylin–eosin (H&E) stain. Images were captured and histo-pathological study was carried out to study the changes in uterine endometrium in bitches.

Peripheral blood samples were collected before operation from each dog in plain vacutainer tube (7 ml) for serum separation. The serum was separated from blood samples by centrifugation for 15 minutes at 2500 rpm and stored at -20°C until analysis. The levels of blood urea nitrogen (BUN), creatinine (CR), albumin, globulin and total protein were estimated as per standard techniques using kits. The collected data were analyzed statistically using completely randomized design and 't' test.

Result and Discussion

The age of affected bitches ranged has ranged between 4½ to 11 years. The highest incidence of pyometra was recorded in bitches >7 years old which concurred with the findings of Koli *et al.* (2017). The ultrasound examination carried out in the affected bitches revealed the presence of uterine exudates such as blood, mucus, pus and cystic endometrial hyperplasia. We were able to clearly evaluate endometrial integrity and variation of uterine wall thickness through ultrasound.

Table 1: Relation between biochemical and histopathological changes with the ultrasonographic uterine wall changes in normal and bitches affected with CEH-pyometra complex

S. No.	Parameters	Control (n=10)	Experimental bitches (n=30)		
			5.1-10.0 mm (n=8)	10.1-15.0 mm (n=13)	15.1-20.0 mm (n=9)
1	BUN (mg/dl)	18.16 ^p ±0.43	22.78 ^q ±1.85	35.03 ^r ±7.45	57.64 ^s ±18.47
2	Creatinine (mg/dl)	0.28 ^p ±0.20	0.91 ^q ±0.39	1.38 ^r ±0.27	1.44 ^r ±0.21
3	Albumin (g/dl)	3.34 ^p ±0.26	3.09 ^p ±0.05	2.96 ^p ±0.46	2.41 ^q ±0.32
4	Globulin (g/dl)	2.54 ^p ±0.04	3.64 ^q ±0.12	4.61 ^r ±0.04	5.67 ^r ±0.22
5	Total protein (g/dl)	5.88 ^p ±0.22	6.73 ^q ±0.21	7.57 ^q ±0.30	8.08 ^r ±0.25
6	Uterine wall thickness (mm)	2.65 ^p ±0.83	8.15 ^q ±1.32	13.01 ^r ±0.94	18.55 ^s ±1.12
7	Histopathological changes	Intact endometrial cells lined by columnar cells	Loss of endometrial surface	Loss of endometrial surface, Enlarged endometrial glands, Fluid accumulation	Loss of endometrium, Enlarged glands, Burst of endometrial glands in various places, chronic cellular infiltration

Means bearing different superscripts (p, q, r, s) between columns differ significantly ($P < 0.05$)

The present study revealed that there was an increase in the uterine wall thickness in bitches affected with pyometra which concurred with the findings of Rubina *et al.* (2002) and Verstegen *et al.* (2008). The control animals had an average uterine wall thickness of 2.65±0.83 mm. In experimental bitches, the ultrasonographic uterine wall thickness was more than 5 mm. Based on the thickness of uterine wall, the experimental animals were divided into three groups as 5.1-10.0, 10.1-15.0 and 15.1-20.0 mm, respectively and the average uterine wall thickness in these groups was 8.15±1.32, 13.01±0.94 and 18.55±1.12 mm, respectively with a significant difference ($P < 0.05$) between them. As the uterine wall thickness increased, we observed a proportionate increase in the serum biochemical profile. The BUN and creatinine levels ranged from 22.78±1.85 to 57.64±18.47 mg/dl and 0.91±0.39 to 1.44±0.21 mg/dl which concurred with the results of Gupta *et al.* (2013) and Lakshmikanth *et al.* (2016). We observed a significant difference between control and experimental bitches in BUN and creatinine levels. There was a gradual elevation in the BUN and creatinine levels and with increase in the thickness of uterus that showed a statistically significant difference which concurred with the results of Manokaran *et al.* (2014). BUN and creatinine, both are catabolic waste products which were excreted through kidneys. Both are indicators of glomerular filtration rate. The concentration of both indicates the extent of damage to kidneys and their concentration increases



according to severity of infection which in turn reflected on thickness of uterine wall (Manokaran *et al.*, 2014).

The albumin, globulin and total protein levels also increased in relation to severity of pyometra and the values obtained in the study coincided with the previous reports of Hagman *et al.* (2009) and Gupta *et al.* (2013). The higher total protein, globulin and albumin levels observed along with the increased thickness of uterine wall in the present study could be due to an acute phase reaction and synthesis of antibodies in response to bacterial infection as mentioned by Hagman (2004). The pre-operative mean serum total protein concentrations was significantly ($P < 0.05$) higher in pyometric bitches (6.73 ± 0.21 to 8.08 ± 0.25) than normal bitches (5.88 ± 0.22). The mean plasma albumin concentration of pyometric bitches was lower (2.41 ± 0.32 to 3.09 ± 0.05 g/dl) than normal bitches (3.34 ± 0.26) whereas the globulin level was higher (3.64 ± 0.12 vs 5.67 ± 0.22 g/dl) than in healthy bitches (2.54 ± 0.04). The hyperproteinaemia in pyometric bitches could be due to elevated globulin component. The observations of hyperproteinaemia, hyperglobulinemia and hypoalbuminemia observed were concurred with findings of Dabhi and Dhami (2006) and Gupta *et al.* (2013). These alterations occur due to loss of albumin via the damaged kidneys and increased production of 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).

The histopathological changes were proportionally higher (severe) in relation to the thickness of ultrasonographic thickness of uterine wall. During histopathological examinations, the endometrium was intact and was lined by columnar cell in control bitches. In the experimental group, the histopathological changes varied according to the thickness of the uterus. The bitches with an average of 8.15 mm (5.1-10 mm group) uterine wall thickness, showed uniform endometrial surface in ultrasonography whereas in histopathology we noticed that there was a loss of endometrial surface. In bitches with 13.01 mm (10.1-15.0 mm group) average thickness, irregular uterine wall was observed in ultrasonography. These bitches had loss of endometrial surface and enlarged endometrial glands with fluid accumulation in histopathology.

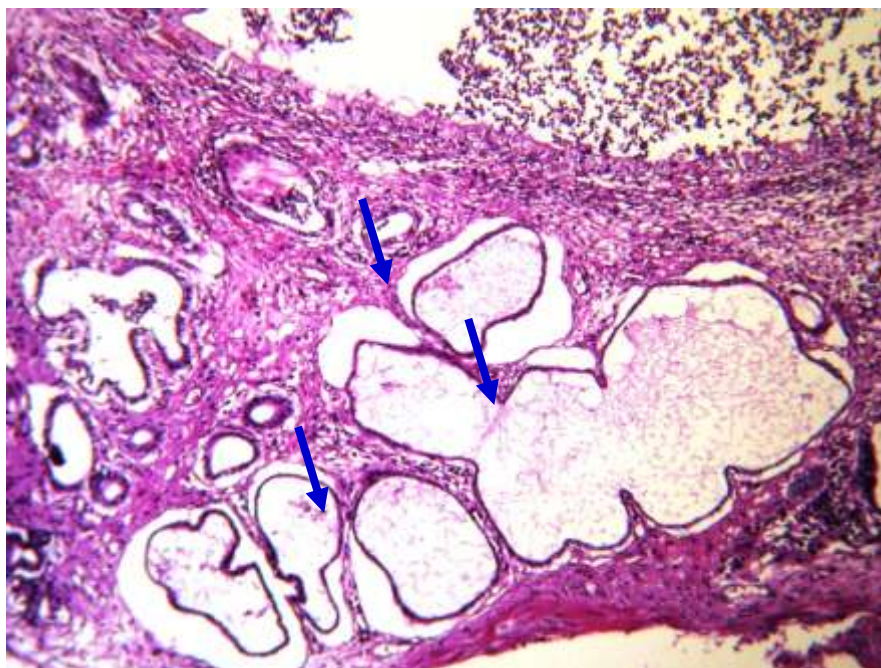


Fig.1: Enlarged endometrial glands with fluid accumulation in 10.1-15 mm uterine wall thickness (Scale bar: 40X)

In bitches with 18.55 mm (15.1-20.0 mm group) thickness irregular endometrial wall with more thickness was observed during ultrasonography which had burst endometrial glands during histopathological examination. The above findings on the ultrasonographic and histopathological changes were in accordance with Bigliardi *et al.* (2004) and Manokaran *et al.* (2014). Arnold *et al.* (2006) reported that the presence of glandular cysts indicated the severity of the disease for which the ovariohysterectomy could be the choice of treatment.

Conclusion

From the above study it was concluded that-

1. Ultrasonography will be a definite, useful and reliable diagnostic tool to detect the pathological changes in the uterine wall during pyometra.
2. As the thickness of uterine wall increases the biochemical parameters viz. BUN, creatinine, globulin and total protein level increased proportionately and hence the thickness can be used as a tool to indicate the severity of infection.
3. Predominant histopathological changes were observed in the affected bitches when the uterine wall thickness increased above 10 mm.

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