



SONOGRAPHIC AND HISTOPATHOLOGICAL STUDY OF MAMMARY TUMOURS IN FEMALE DOGS

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ABSTRACT

The study was conducted on mammary tumour in ten female dogs which were presented to the Veterinary Clinical Complex of the university. The female dogs were of different breeds with age ranging between 5.0 and 16.5 years (median age 10.7). The ultrasonographic images were used to evaluate the shape, size and echogenicity of mammary lesions. In ultrasonogram, benign tumours were homogenous in their internal echogenic pattern i.e. hypoechoic. Ultrasonograms of malignant tumour had many pockets like structure of variable sizes having hyperechoic boundary. Excisional biopsy was taken after the surgery of these cases. Of the clinical 10 cases, 6 were of benign whereas the rest cases were of malignant category. Due to the request of owners of the animals, surgeries were performed and tumours were confirmed by histopathology. There were four cases of adenocarcinoma, two cases each of benign mixed tumours and myoepithelioma and one case each of adenoma and myxoma. Skin sutures were removed after 12 days. Post-operative care was done with antibiotics (ceftriaxone + tazobactam), analgesics (meloxicam), B-complex and vitamin C injections for 5 days. The study suggested that ultrasonography alone is sufficient to diagnose benign and malignant mammary tumors.

Keywords: Dog, histopathology, malignant, mammary tumour, ultrasonography

INTRODUCTION

Mammary tumours are one of the most common oncological diseases diagnosed in female dogs (Stan *et al.*, 2020). These tumours account for up to 52% of all tumours in the female dogs (Kumar and Parashar, 2020). Mammary gland tumours affect middle aged and old female dogs that are sexually intact (Cassali *et al.*, 2011). Canine mammary tumours are commonly presented as circumscribed nodules with variable size, consistency and mobility to the skin and muscle. These can also be associated with skin ulceration and local inflammatory reactions. Multiple tumours are frequently observed in a single mammary gland or may involve multiple mammary glands simultaneously (multicentric tumours) and can be of different histological type (Misdorp *et al.*, 1999). Mammary tumours are a major health threat to women and female dogs. In both, metastasis of primary tumour to distant organ is the most common cause of tumour-related death (Klopfleisch *et al.*, 2010). The metastasis makes female dogs more prone to anaesthetic and surgical risk. The majority of dogs with mammary tumours are clinically healthy at the time of diagnosis and the tumours can be identified by the owner or a professional during a routine physical examination

(Sorenmo, 2003). Biopsies were not made due to the invasiveness of this procedure and because it may lead to inappropriate diagnostic delay (Masciadri and Ferranti, 2011). In this context, non-invasive pre-surgical differentiation between neoplastic and non-neoplastic benign lesions can prevent unnecessary invasive surgery. For diagnosis, ultrasonography can be used to differentiate the mammary tumours into benign or malignant (Nyman *et al.*, 2006) and can become a valuable tool to characterize physiological changes in mammary tissue (Payan and Martin, 2008). Surgical treatment is the first choice for all female dogs with mammary tumours (Sorenmo *et al.*, 2013). The histological classification of mammary tumours can become a valuable tool for prediction of their biological behaviour (Tanno *et al.*, 2006). After deciding about the type of tumour and extent of malignancy, the clinician can decide about the suitable line of treatment. Use of mitotic inhibitor alone e.g. vincristine sulphate is not so fruitful at this very stage as it is observed in cases of venereal granulomas. Therefore, surgical intervention is the only option with the clinician to handle mammary tumour in female dogs that is more suitable in early stage of the disease. Therefore, the study was envisaged with the objective to correlate the findings of ultrasonography of mammary tumours with their post-surgical histopathology.

MATERIALS AND METHODS

Description of the cases

The study was conducted on 10 cases of mammary tumours in clinical cases of female dogs, which were brought to the Veterinary Clinical Complex of Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar, Haryana (India). These dogs belonged to the following breeds: German Shepherd (n = 5; case No. 12-3755, 4-7542, 6-9244, 6-9303 and 6-9627), Mongrel (n = 2; case No.3-5274 and 4-7287), Spitz mixed (n = 1; case No.3-5690), Spitz (n = 1; case No. 9-2208) and Rottweiler (n = 1; case No. 4-7855). The age of female dogs was in between 5 to 16.5 years (median age, 10.7 years) and all were intact.

Different diagnostic methods namely, ultrasonography and histopathology were performed to differentiate the malignant from benign tumours. The surgeries were performed on the requests of animal owners. For management of these conditions, surgery was performed under general anaesthesia. Thoracic and abdominal radiography revealed no metastatic changes in other visceral organ which could be due to the early identification of tumorous growths. Pre-operated mammary tumour cases were stabilized by giving balanced electrolyte fluid and vitamins. The female dogs were injected with atropine sulphate @ 0.04 mg kg⁻¹ bw subcutaneously and later with xylazine HCl @ 0.8 mg kg⁻¹ bw. Injections of ketamine HCl @ 7 mg kg⁻¹ bw and diazepam @ 0.05 mg kg⁻¹ bw were given with Ringer's lactate drip to maintain the surgical stage of anaesthesia. The post-operative care was advised with inj. ceftriaxone + tazobactam @ 15-25 mg kg⁻¹ bw i/m, inj. meloxicam @ 0.5 mg kg⁻¹ bw i/m, inj. tribivet® (Intas Pharmaceutical Ltd; vitamin B-complex) 1 mL i/m and inj. C-Mac® (Macknor Life Sciences Pvt. Ltd.) 2 mL i/m for 5 days. Daily antiseptic dressing was advised and the skin sutures were removed on day 12 post-surgery.

Ultrasonography

A 7.5 MHz curvilinear ultrasound transducer (Toshiba Diagnostic ultrasound system, model SSA-530A) coupled with an ultrasound gel between the probe and skin was used. The ultrasonographic images were used to evaluate the shape, size and echogenicity of mammary lesions and their relationship with surrounding tissues. The ultrasonograms of benign tumours were homogenous in their internal echogenic pattern i.e. hypoechoic, with hyperechoic boundary. Similarly, ultrasonograms of intact non-pregnant cats appeared with a regular hypoechoic texture and showing a thickness of less than 2.0 mm (Payan and Martin, 2008).

RESULTS AND DISCUSSION

Ultrasonograms of malignant tumours had many pocket-like structures of varying sizes with heterogeneous echotexture and hyperechoic boundary. The contents of some pockets were hyperechoic, while others anechoic (Fig. 1). Out of the 10 cases examined by ultrasonography, six (12-3755, 3-5274, 3-5690, 4-7855, 6-9244 and 4-7542) were confirmed benign and four as malignant (9-2208, 4-7287, 6-9303 and 6-9627). Malignant tumours had heterogeneous echotexture and mixed tumour component which is in line with Guedes *et al.* (2020). Thus, sonography is a diagnostic method that may help in differentiating the benign and malignant solid tumours.



Fig. 1: The ultrasonogram of benign mammary gland tumour in female dog (case No. 9-2208) showing hyperechoic boundary (red arrow) with hypoechoic granules (blue arrow)

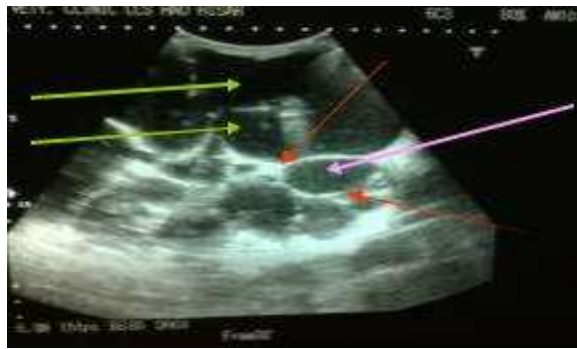


Fig. 2: The ultrasonogram of mammary gland in female dog (case No. 12-3755) showing multiple lobules divided by hyperechoic septa (red arrow) and the lumen of some lobules anechoic (green arrow) and others hyperechoic (pink arrow).

Histopathology

The female dogs were observed closely for the presence of growth and the shape, size, consistency and colour of cut surfaces of growth were recorded. The representative tissue sample were fixed in 10% formalin and processed for histopathology and stained with haematoxylin and eosin as per Luna (1968). Histopathological examination revealed four of these cases as adenocarcinoma (case No. 12-3755, 3-5274, 3-5690 and 4-7855), two each as benign mixed tumours (case No. 6-9244 and 4-7522) and myoepithelioma (case No. 9-2208 and 4-7287), and one each as adenoma (case No. 6-9303) and myxoma (case No. 6-9627). The detailed description of each type of tumour is as under: Grossly, the adenoma was nodular, encapsulated and pink in colour. Microscopically, it revealed irregular and enlarged acini with papillary projection. These were mostly lined by single layer of epithelial cells (Fig. 3). Adenoma is a benign neoplasm of well-differentiated epithelial or myoepithelial cells. Tumours composed of well-differentiated epithelial cells were classified as a simple tubular type and should be differentiated from adenosis (Cassali, 2002). In present study, the myxoma was non-capsulated, round in shape and appeared as bunch of grapes. It was soft in consistency, slimy, non-capsulated and of greyish white colour. Microscopically, the neoplastic cells were stellate shaped along with long cytoplasmic processes and had light bluish mass in between the cytoplasmic processes (Fig. 4). In myoepithelioma, neoplastic cells were spindle shaped and irregularly arranged. Adenocarcinoma appeared as soft and cut section revealed a diffuse or lobular structure of homogenous tissue of creamish-white in colour. In two cases, these were irregular in shape, hard and colour was grey or white with yellow spots. Microscopically, the neoplastic cells were anaplastic, characterized by lose of their polarity and hyperchromatic nuclei. Acini revealed many layers of cells which were arranged in tubules of irregular size and often contained detached cells giving appearance of solid masses of cells. Mitotic figures were present. There was necrotic area in between tumour cells (Fig. 5). Simple tubular adenocarcinoma has been

relatively frequent tumour in dogs, the tubular type being predominant and the microscopic structure is dominated by tubular epithelial cells in which pleomorphism and mitotic activity varied from low to high and necrotic foci were frequent (Misdorp *et al.*, 1999). Malignant myoepithelioma appeared as round or ovoid formation up to 4 cm in diameter, of soft consistency, poorly circumscribed and frequently ulcerated. Further, microscopically the cells are arranged in bundles or spirals, with myoid aspect and the loss of lobular structure from it (Cotchin, 1958).

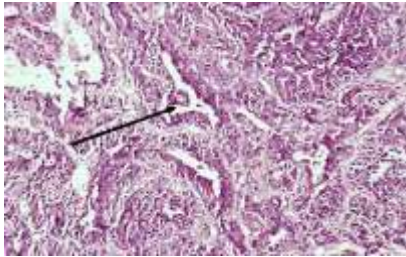


Fig. 3: The microphotograph of mammary gland tissue in female dog (Case No. 6-9303) showing irregular & enlarged acini with papillary projection (black arrow) which were mostly lined by single layer of epithelial cells. Fibrous stroma is seen in between acini (H&E stain) (x200)

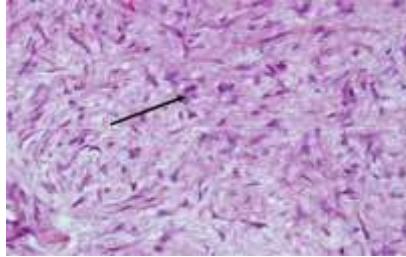


Fig. 4: The microphotograph of mammary gland tissue in female dog (Case No. 6-9627) showing star shaped/ stellated tumorous cells (black arrow) (H&E stain). (x400)

Benign mixed tumours were spherical, ovoid in shape, appeared as pendulous mass, affecting one or more mammary glands. Microscopically, these tumours were comprised of cartilage, myoepithelial cells and myxomatous tissue (Fig. 6). Misdorp *et al.* (1999) reported the benign mixed tumours characterized by benign proliferation of cells that were morphologically similar to epithelial components and

mesenchymal cells that produce cartilage and/or bone and/or adipose tissue, possibly in combination with fibrous tissue. Tumour development time is a subjective tool since some owners are unaware of the precise time when spontaneous neoplasms develop in their dogs. However, a longer mean development time was observed for carcinosarcomas than for carcinomas in mixed tumours, and of these, compared with benign mixed tumours, carcinosarcomas showed a positive correlation between progression time and malignant transformation (Nunes *et al.*, 2019).

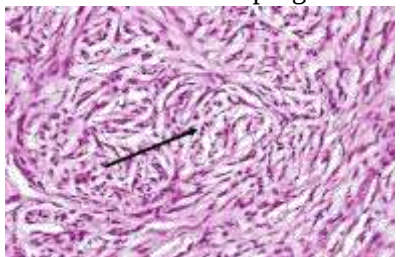


Fig. 5: Microphotograph of mammary gland tissue in female dog (Case No. 12-3755) also showing spindle shaped and irregularly arranged tumorous cells (black arrow) (H&E stain) (x100)

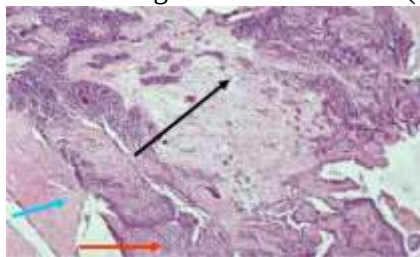


Fig. 6: Microphotograph of mammary gland tissue in female dog (Case No. 6-9244) also showing cartilage tissue (black arrow), myoepithelial and epithelial tissue (red arrow) with abundant fibrous tissue (blue arrow) (H&E stain) (x200)

In present study, the incidence of benign tumours was 60%. Brearley (1989) in female dogs observed the incidence of mammary tumours as 50% of all the tumours; of which about 60% were benign and 40% malignant. According to the histologic diagnosis, 41-53% mammary tumours in female dogs are malignant (Lana *et al.*, 2007). In present study, mainly posterior mammary gland was involved. Higher tumour incidence in posterior

mammary gland pair was correlated with the higher gland volume and abundant secretion during the lactation period (O'Keefe, 1995). In canine mammary tumours, ultrasonographic characteristics appear to be correlated with histopathologic changes (Nyman *et al.*, 2006). Malignant nodules are often characterized by poorly defined margins and irregular borders. On the other hand, benign lesions are often well differentiated from the surrounding tissue by a well-defined, circumscribed margin and border. The study suggests that ultrasonography may have an important role in the

evaluation of mammary tumours in dogs, particularly in the evaluation of tissue composition and tumour echotexture. The ultrasonography as a valuable diagnostic tool can help in differentiation of benign or malignant tumours. Furthermore, the predictions with ultrasonography can be confirmed with histopathology. Conclusively, ultrasonography itself alone is sufficient to diagnose benign and malignant mammary tumors.

Ethical statement: The study was conducted on the clinical cases presented to Veterinary Clinical Complex of Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar (Haryana) India. There was no need of ethical approval from IAEC.

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