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Doxorubicin and cyclophosphamide chemotherapy, with or without surgical excision, for management of canine mammary tumours

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The present study was carried in 18 canine mammary tumour cases. The dogs were divided into three groups viz., C, A, and S, as per the treatment given. The animals of group S were treated with surgery alone (simple mastectomy, enblock dissection and lumpectomy); in group A, the animals were treated with surgery and adjuvant chemotherapy with a combination of doxorubicin (25 mg/m² i.v.) and cyclophosphamide (30 mg/m²), given at 10 days interval for three times; and group C animals were treated with chemotherapy alone. Lateral thoracic radiography was performed to evaluate the distant pulmonary metastasis. Ultrasonography was done in all the cases to determine the echogenicity of tumour mass. Histopathological evaluation revealed majority of the tumours to be malignant. Combination of doxorubicin and cyclophosphamide was found to be effective as an adjuvant therapy in the treatment of mammary tumours, and this combination increased the longevity and survival of animals.

Key words: *Canine mammary tumour, Chemotherapy, Cyclophosphamide, Doxorubicin*

Treatment of canine mammary neoplasms (CMNs) mainly involves surgery (viz., lumpectomy, simple mastectomy etc.), radiotherapy, chemotherapy, or their combinations. The surgical approach is often employed in conjunction with chemotherapeutic protocols to enhance treatment efficacy and improve clinical outcomes. Chemotherapy is supposed to treat systemic or unknown metastasis of mammary tumours.

The primary goal of adjuvant chemotherapy is to remove the occult metastasis that is responsible for late recurrence (Giuliano, 1991). Bitches with malignant neoplasms can benefit from combining antineoplastic chemotherapy protocols and antiangiogenic strategies. Doxorubicin and cyclophosphamide as adjuvant chemotherapeutic agents may show promising results in treatment of CMNs. The present study was conducted to evaluate the antineoplastic efficacy of doxorubicin and cyclophosphamide in canine mammary tumour.

Materials and Methods

The present study was conducted on clinical cases of mammary tumours to evaluate the antineoplastic efficacy of doxorubicin and cyclophosphamide in three groups of six animals each. These groups were designated as S (surgery alone), A (adjuvant chemotherapy) and C (chemotherapy alone).

The chemotherapeutic regimen in group A and group C was:

a. Injection doxorubicin hydrochloride @ 25 mg/m² surface area, i.v., once on day one, diluted with normal saline.
b. Tablet cyclophosphamide @ 30 mg/m² once a day for 4 days, one day after doxorubicin therapy. This constituted one cycle of chemotherapy and three such cycles were administered at 10 days interval.
c. Injection dexamethasone @ 0.4-2.0 mg/kg i.v., and chlorpheniramine maleate @ 2 mg/kg i.m., were also given to the animals to prevent allergy and any other side effects, before administration of doxorubicin hydrochloride.

In groups S and A appropriate surgery was performed to remove the tumorous growth.

Clinical evaluation of the dogs was performed, and general condition, breed, age, sex, body weight, duration of clinical signs, reproductive history, recurrence following previous excision or chemotherapy, size of neoplasm with broadest diameter, nipple deformities, ulceration, inflammation, pruritis or infection, discharge from teat, number of glands involved, rate of growth and location of the tumour, consistency of tumour mass and its attachment to underlying or overlying tissues, were recorded.

Ultrasonographic examination of neoplastic mass was done before starting the surgery or chemotherapy in all animals. Subsequently, it was repeated after 10 and 20 days to determine their echogenicity as well as response to the chemotherapy in animals of group C. Lateral radiograph of thorax was done on 0, 10 and 20 days to detect any metastatic lesions in lungs. Haemato-biochemical analysis was carried out at 0, 10 and 20 days using standard procedures. Haemoglobin concentration and

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differential leukocyte count (DLC) were carried out in blood samples. Serum creatinine and blood urea nitrogen (BUN) were estimated using standard commercial kits. Histopathological examination of excised tumour mass was carried out in group S and A. The tissue was fixed in 10% neutral buffer formalin (NBF) and processed for histopathology using standard Haematoxylin and eosin (H&E) stain. Fine needle aspiration biopsy technique was used for the cytologic examination of 6 canine mammary tumours (Group C) through Geimsa staining (Sontas *et al.*, 2009).

Results and Discussion

In this study, only one gland was grossly involved in the majority of cases (66.67%). Similarly, Gupta *et al.* (2012) have reported that in most of the mammary tumour cases only one gland was involved. Fifth pair and fourth pair of mammary glands were equally affected (30.76% each), followed by third pair (20.83%), second pair (19.23%), and no tumour mass was recorded in the first pair of mammary glands. Because the fifth pair of mammary glands are larger and include more mammary tissue, they may be more susceptible to a wider range of physiological alterations that could lead to neoplasms (Lather *et al.*, 2017). Majority of mammary tumour cases (66.66%) had a slow rate of growth and only 33.33% cases were fast-growing. The rate of tumour growth does have prognostic significance and generally slow growth of the tumour is associated with a better prognosis (Theilen and Madewell, 1979).

Seven (38.89%) of the affected canines had a duration of illness between 3 and 6 months, five (27.78%) had a duration of 0 to 3 months, 4 (22.22%) had a duration of more than 9 months, and 2 (11.11%) had a duration of 6-9 months. Most of the mammary tumour cases (94.44%) were presented for the first time, and the rest 5.55% were recurrence of the previous episode. The recurrence was observed more in the animals of the group S, suggesting that surgery alone was not an optimal treatment modality for the mammary tumour.

Most of the mammary tumours were found to be hard in consistency (50%) followed by firm (44.44%) and soft (5.55%) consistencies. Most of the tumours (61.11%) presented were attached to the skin, while the rest (38.89%) were found attached to both the overlying skin and the underlying body wall. About 22.22% of the mammary tumours had ulceration, 16.67% had inflammation, and 22.22% had an infection, while the rest of 38.89% tumours did not show any additional external lesions. Nipple deformities were observed in 5 (27.78%) cases, whereas the rest of the cases (72.22%) did not show any kind of deformity. In 13 (72.22%) cases no abnormal discharge was observed from affected teats, whereas in 4 cases cystic, and in one case milky discharge was observed.

Radiography

Lateral thoracic radiography of all the dogs was performed on days 0, 10 and 20, to evaluate the effect of treatment on distant metastasis (Figs. 1-2). Metastasis of lungs is common because of high blood flow and capillary network that provoke slower circulation. In the present study, thoracic radiography of 5 out of 18 cases of the mammary tumour revealed soft tissue density in the lung area indicating pulmonary metastasis, while the rest 13 cases revealed no evidence of lung metastasis. In the present study, the radiographic examination on day 20 of chemotherapy treatment suggested that there was a regression in pulmonary metastasis in response to treatment with a combination of cyclophosphamide and doxorubicin in dogs with a malignant mammary tumour, which is in accordance with Todorova *et al.* (2005).

Ultrasonography

In the present study, ultrasonography of the

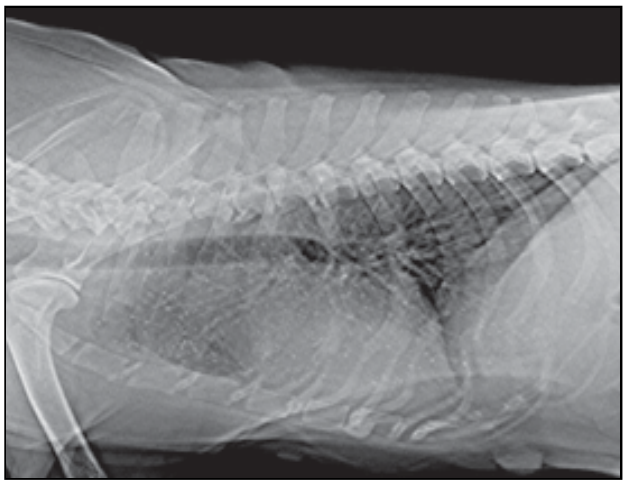

Fig. 1: Lateral thoracic radiograph of non-descript dog treated with chemotherapy at 0 day showing radio-dense metastatic foci spread throughout the lung.

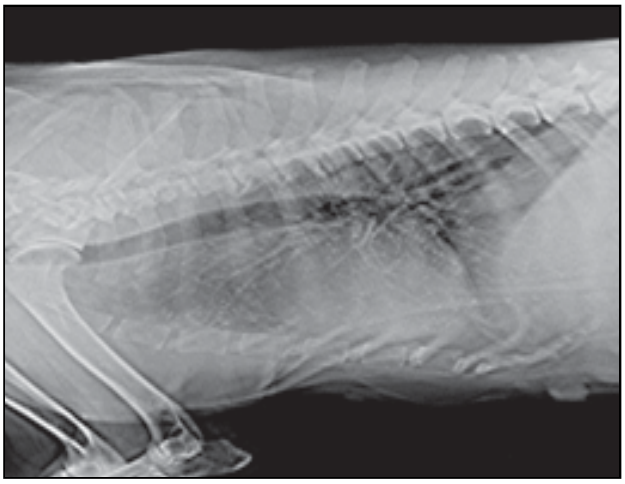

Fig. 2: Lateral thoracic radiograph of the same dog at 20 days showed very less number and less radio-dense metastatic tumour nodules in comparison to day 0 and 10.

affected gland showed that 72.22% of the neoplasms were homogenous in echotexture, and 27.78% were heterogenous (Figs. 3 and 4). It was also observed that 50% of the tumours were non-cystic, 5.55% was cystic and rest 44.44% were of mixed nature. In 61.11% of cases, tumour had irregular margins and were polymorphic in shape, while 38.89% had well defined margins. Out of the 18 mammary tumours, 83.33% had distal acoustic enhancement, while 16.67% showed distal acoustic shadowing.

Nyman *et al.* (2005) reported that irregular borders, invasive tumour growth, high vascularity and heterogeneous echotexture were indicative of malignant growth. Sharma *et al.* (2018) revealed that benign and malignant tumours could not be differentiated based on clinical examination or ultrasonography, as some of the tumours with a firm attachment to the skin and underlying tissues, and those with heterogenous echotexture mixed with anechoic cavities, were both benign as well as malignant. However, all the mammary tumours having homogenous echotexture on ultrasonography were benign.

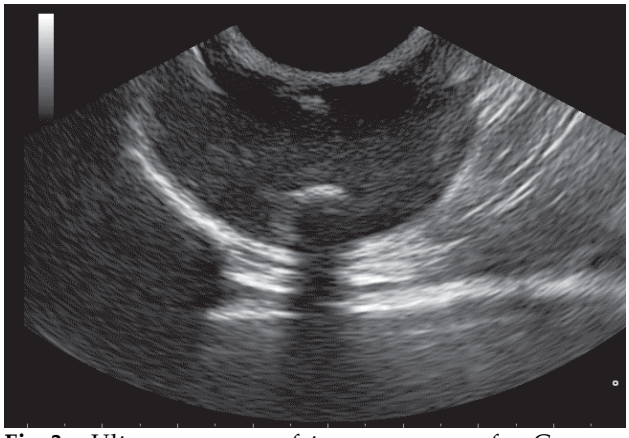

Fig. 3: Ultrasonogram of tumour mass of a German shepherd dog aged 9 years showing well defined border, central calcification, acoustic shadow, and heterogenous echotexture.

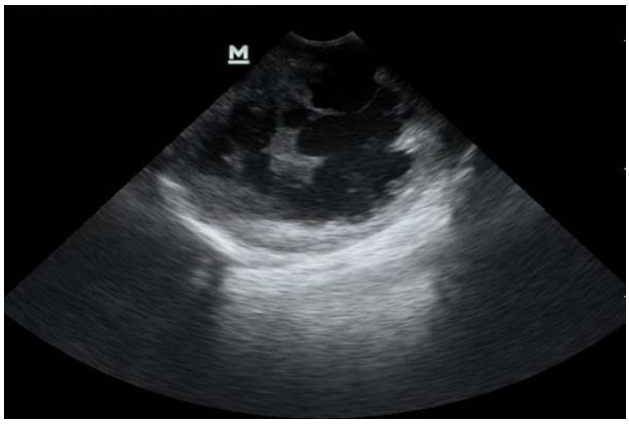

Fig. 4: Ultrasonogram of tumour mass of non-descript dog aged 9 years showing well defined border, acoustic enhancement, cystic and heterogenous echotexture.

Haemato-biochemical parameters

No significant variation (P>0.05) was observed in the haemoglobin concentration at different time intervals in any of the groups, and the values remained close to lower normal levels. Neutrophil counts decreased significantly (P<0.05) in groups C and A, after chemotherapy. Similar reductions were reported during vincristine and doxorubicin therapy (Kumar *et al.*, 2018). Lymphocyte count increased significantly (P<0.05) on day 20 in groups C and A, but remained within normal limits. Comparable findings were also noted by Khan *et al.* (2017) and Kumar *et al.* (2018). Eosinophil counts did not vary significantly at any time period, in any of the groups.

Blood urea nitrogen (BUN) varied non-significantly at different time intervals, in all groups. Gandhimati *et al.* (2011) reported no significant changes in BUN in vincristine- and doxorubicin-treated dogs with transmissible venereal tumour. BUN is a key indicator of renal function in dogs. Increases may result from reduced glomerular filtration or elevated protein catabolism due to tumour necrosis or neoplastic metabolism, while elevated serum creatinine reflects increased catabolic activity (Kumar *et al.*, 2018). Serum creatinine levels also did not vary significantly (P>0.05) at any time interval, in all groups.

Fine needle aspiration cytology (FNAC) and histopathology

In the present study, samples from 6 dogs were collected for FNAC. One of the six samples was classified as insufficient and inadequate for diagnosis, and the remaining 5 samples were diagnosed as malignant tumours. The epithelial cells in malignant tumour sections showed pleomorphic, basophilic and large nucleus with or without prominent nucleolus. In some cases, a mixed population of cells were also seen. Out of the 12 cases of mammary tumour examined with histopathology, 10 cases were malignant, whereas, 2 were benign (Figs. 5-6). Fibroadenoma (2) were recorded as the only benign

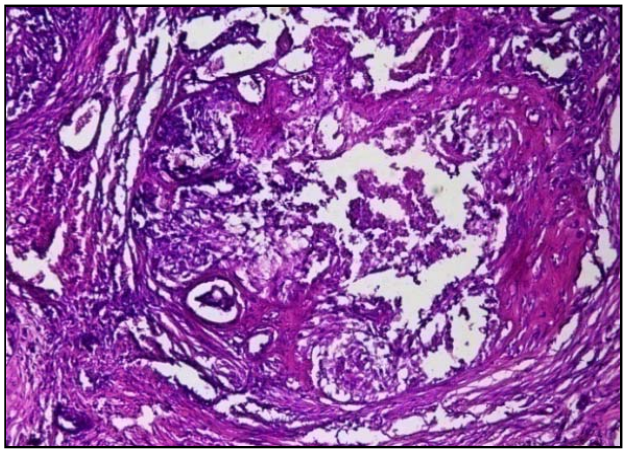

Fig.5: Mammary gland tumour in dog: the tubular to papillary proliferation of glands surrounded with fibrous stroma suggesting carcinoma (H&E × 200).

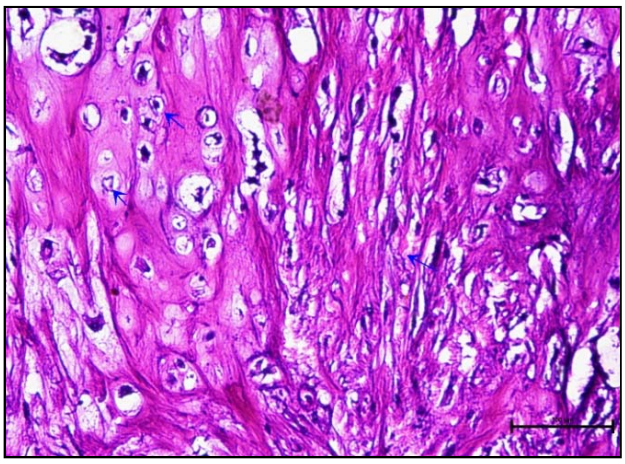

Fig. 6: Mixed mammary gland tumour in dog: neoplastic cells showing hyperchromatic nuclei and bi/multinucleate appearance with irregular outline. The fibrous tissue is present between basophilic chondroid materials (H&E × 200)

tumour type. Similar findings were recorded by various researchers (Shekar *et al*., 2001; Feliciano *et al*., 2017). Mixed mammary tumours were the most common (6) type, followed by adenocarcinoma (2) and fibrosarcoma (2).

Surgery

Surgery was performed on 12 clinical cases of mammary neoplasm. In 6 cases surgery alone was done, and in 6 cases, surgery along with chemotherapy was performed. The various forms of surgical excisions performed were enblock dissection (n=6), simple mastectomy (n=3), and lumpectomy (n=3) depending on the size and volume of the tumour mass. Affected mammary gland and their draining lymphatics were removed during enblock dissection. The tumour and affected gland were removed in a simple mastectomy. Enblock resection showed good results in the present study as compared to simple mastectomy, as the recurrence was less. The recurrence was seen in only one case operated by enblock resection. This is due to the belief that there might be micrometastases in lymph nodes or other mammary lobes (Gýlbertson *et al*., 1983). In contrast, Misdrop and Hart (1979) reported that the animals treated by enblock dissection and mastectomy had similar post-surgical survivability.

Chemotherapy

In the present study, adjuvant chemotherapy with doxorubicin and cyclophosphamide was done in 6 malignant mammary tumours (adenocarcinoma, fibrosarcoma and mixed type), and showed good results. In group A, the first case showed adenocarcinoma with high neutrophils and lung metastasis; the case survived. The second and third cases were mixed mammary tumours; one with marked fibrosis and no metastasis, the other with low Hb and lung metastasis with recurrence; both survived. The fourth case was fibrosarcoma with high neutrophils, low Hb, and lung metastasis; the case survived. The last two cases were mixed mammary tumours with high neutrophil and monocyte counts, no metastasis, and both survived.

Chemotherapy alone was done with doxorubicin and cyclophosphamide in 6 animals (group C) having tumour diameter ranging from 2-6 cm in which none of the animals showed complete regression of tumour mass. In two cases, having diffused type of mammary tumour, significant regression was observed at 4 weeks after the last chemotherapy. In this group, one animal died after the last follow up and in another case, recurrence was seen.

None of the animals of group C showed complete regression of tumour mass indicating that surgery should be done along with adjuvant chemotherapy. Hershey *et al.* (1999) and Khan *et al.* (2017) have reported that adjuvant chemotherapy with doxorubicin was more effective than surgery alone or chemotherapy alone. Contrary to this, Simon *et al.* (2006) reported that doxorubicin administered in addition to surgery did not significantly increase the overall survival, recurrence-free interval and metastasis-free interval.

Clinical toxicity

In the present study, out of 12 cases subjected to chemotherapy, 9 cases showed no signs of clinical toxicity, whereas in one animal the value of BUN and creatinine increased after administration of 2nd session of chemotherapy, as a result of which the animal showed the neurological signs (circling and head pressing), vomiting, anorexia, weight loss, and at last the animal died. Two animals showed mild toxicity viz. vomiting, alopecia, anorexia, diarrhoea, and weight loss but survived.

Moderate neutropenia and clinical symptoms such as lethargy, anorexia, vomiting, diarrhoea and fever were associated with the toxicity of doxorubicin and cyclophosphamide therapy (Sorenmo *et al*., 1993; Todorova *et al*., 2005).

Follow-up

All 18 cases reported for the follow-up in the present study, of which one dog died, 3 were reported living with recurrence. Out of six animals receiving adjuvant chemotherapy in the present study all of them survived (100%). None of the animals receiving adjuvant chemotherapy in the present study were reported living with recurrence.

Of the 6 animals which received chemotherapy alone, one died and 2 showed partial tumour regression. Three animals showed no evidence of regression, and no complete regression was observed in any animal. Todorova *et al.* (2005) found in two dogs, the tumour growth recurred about 1.5 months after the chemotherapy. Of the 6 animals subjected to surgery alone, 2 reported back for living with recurrence. Thus, the overall survival rate was 66.67% (disease free survival).

To conclude, the combination of doxorubicin and cyclophosphamide was found to be effective as adjuvant therapy in treatment of mammary tumour and this combination increased the longevity and survival of animals.

References

Feliciano, M.A., Uscategui, R.A., Maronezi, M.C., Simões, A.P., Silva, P., Gasser, B., Pavan, L., Carvalho, C.F., Canola, J.C. and Vicente, W.R. 2017. Ultrasonography methods for predicting malignancy in canine mammary tumours. PloS One **12:** e0178143.

Gilbertson, S.R., Kurzman, I.D, Zachrau, R.E., Hurvitz, A.I. and Black, M.M. 1983. Canine mammary epithelial neoplasms: biologic implications of morphologic characteristics assessed in 232 dogs. Vet. Path. **20**:127-142.

Giuliano, A.E. 1991. Breast. *In*: Current Medical Diagnosis and Treatment, Schroeder, S.A., Krupp, M.A. and Tieney, L.M. (Eds), 30th edn. Prentice Hall International (UK) Ltd., London. pp 486-503.

Gupta, K., Sood, N., Uppal, S., Mohindroo. J., Mahajan, S., Raghunath, M. and Singh, K. 2012. Epidemiological studies on canine mammary tumour and its relevance for breast cancer studies. J. Pharm. **2:** 322-333.

Hershey, A.E., Kurzman, I.D. and Forest, L.J. 1999. Inhalation chemotherapy for microscopic primary and metastatic lung tumours. Proof of prenatal dogs with spontaneously occurring tumours as a mode. *Clin. Cancer Res.* **5**: 2653-2659.

Khan, G.J., Thorat, M.G., Raulkar, R.V., Upadhye, S.V., Jadhao, P.T., Chepte, S.D. and Deshmukh, S.G. 2017. Surgico-chemotherapeutic management of mammary tumour in canine. Int. J. Sci. Environ. Technol. **6**: 3154-3159.

Kumar, V.A., Kumari, K.N., Kumar, K.S. and Gireesh, V. 2018. Effect of vincristine chemotherapy in TVT affected dogs. J. Innov. Pharm. **7**: 163-166.

Lather, D., Gupta, R.P. and Jangir, B.L. 2017. Pathological and immunohistochemical studies in mammary gland tumours affecting male dogs. Indian J. Vet. Pathol. **41**: 89-93.

Misdrop, W. and Hart, A.A.M. 1979. Canine mammary cancer. 1. Prognosis II Therapy and causes of death. J. Small Anim. Pract. **20**: 385-404.

Nyman, H.T., Kristensen, A.T., Lee, M.H., Martinussen, T. and McEvoy, F. 2005. Characterisation of canine superficial tumours using gray-scale B-mode, color flow mapping and spectral Doppler ultrasonography - a multivariate study. Vet. Radiol. Ultrasound **47**: 192-198.

Sharma, N., Gupta, A.K., Bhat, R.A., Yatoo, M.I. and Parray, O.R. 2018. Epidemiology and treatment of canine mammary tumours in Jammu region of India. J. Dairy Vet. Anim. Res. **7**: 59-62.

Shekar, C.S., Vijarasarathi, S.K., Rao, S. and Gowda, R.N. 2001. Haematological screening in dogs with mammary tumours. Indian Vet. J. **78:** 163-164.

Simon, D., Schoenrock, D., Baumgärtner, W. and Nolte, I. 2006. Postoperative adjuvant treatment of invasive malignant mammary gland tumours in dogs with doxorubicin and docetaxel. J. Vet. Intern. Med. **20**: 1184-1190.

Sorenmo, K.U., Jeglum, K.A. and Helfand, S.C. 1993. Chemotherapy of canine hemangiosarcoma with doxorubicin and cyclophosphamide. J. Vet. Intern. Med. **7**: 370-376.

Theilen, G.H. and Madewell, B.R. 1979. Veterinary Cancer Medicine, 1st edn. Lea and Febiger, Philadelphia. pp 192-203.

Todorova, I., Simeonova, G., Simeonov, R. and Dinev, D. 2005. Efficacy and toxicity of doxorubicin and cyclophosphamide chemotherapy in dogs with spontaneous mammary tumours. Trakia J. Sci. **3**: 51-58.