CLINICAL STUDY FOR DIAGNOSIS AND TREATMENT OF CANINE MAMMARY NEOPLASMS (CMNs) USING DIFFERENT MODALITIES

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ABSTRACT
The study was performed on 40 canines presented with the history of any swelling or growth in one or all of the mammary glands. Signalment and history was recorded properly and then the animals were subjected to detailed physical examination. Fine needle aspiration biopsy (FNAB) of the tumorous mass was taken to give preliminary diagnosis. The distant metastasis was confirmed via lymph node palpation, chest radiography and ultrasonography. Based on treatment regimen followed the animals were divided into two groups: group I (n= 29) in which only surgery was performed and group II (n= 6) in which surgery as well as chemotherapy was performed while remaining 5 animals were not given any treatment. Different haematological (Hb, TLC, DLC and PCV) and biochemical (BUN, CRtN, ALT, AST, ALKP and TP) were recorded preoperatively, postoperatively and after chemotherapy at required intervals. In group II animals chemotherapy was started 15 days after surgery and the regimen followed was inj. Vincristine @ 0.5mg/m²BSA and inj. Methotrexate @ 9-15mg/m²BSA, i.v. on day 1 followed by tab. Cyclophosphamide @ 50mg/m² po from 2nd to 5th day. Maximum (57.5%) no. of cases were recorded in Samoyed breed and the median age of affected animals was 10.8 years (2-15). Diagnostic accuracy, sensitivity and specificity of FNAB were 77.14%, 78.78% and 100% respectively. 74.28% of cases showed pulmonary metastasis in lung radiography. All haematological and biochemical parameters remained within normal range during the course of therapy except significant decrease in ALKP values after surgery in both groups. Majority (96.96%) of the neoplasms were malignant and there was no significant difference in the mean survival time and survival rate after surgery in both groups and among different surgical technique. The most significant finding was that all the live animals in group II were less than 8 years of age suggesting that the combination used in the present study was safe and effective in comparatively young animals.

Key words: Canines, Chemotherapy, CMNs, Tumors.

INTRODUCTION
Among domestic animals the neoplastic conditions are more common in dogs. Canine mammary neoplasms (CMNs) are second most commonly occurring tumors next only to skin tumors (Moulton 1990) accounting to approximately 50% of all tumors in female dogs of which 40 to 50% are malignant (Sorenmo 2003). The treatment of CMNs forms the most challenging aspect of companion animal practice and has been controversial subject for decades. Classical modalities of cancer therapy include surgery, radiation therapy, chemotherapy, cryosurgery, immunotherapy, hyperthermia and use of biological response modifiers. Though surgery is the treatment of choice, its value reduces when it is used alone for certain tumors which carry high risk of metastasis (Theilen and Madewell 1979). Adjuvant chemotherapy which eliminates the occult microscopic metastasis is now a days very common in use. Not a single antineoplastic agent has been reported to have 100% efficacy in managing canine neoplasms. Therefore, the emerging trend is to use a combination of drugs for treatment of CMNs. Adjuvant chemotherapy using different combinations of antineoplastic drugs have also been found effective in treatment of human breast cancer (Rivkin et al. 2003). Use of such drugs for treatment of canine neoplasms has also been suggested by...
Helfand (1990). So the present study was designed
to study the efficacy of adjuvant chemotherapy using
combination of vincristine, methotrexate and
cyclophosphamide and to compare the results of
surgery alone and adjuvant chemotherapy in treating
canine mammary neoplasm cases.

MATERIALS AND METHODS
The study included 40 cases of canine
mammary neoplasms presented in Teaching
Veterinary Hospital, GADVASU-Ludhiana for
treatment. Different clinical parameters like
signalment, general body condition, reproductive
history, duration of clinical signs, number of
mammary glands involved, location, size,
consistency and attachment of tumorous mass, any
discharge or nipple deformity, mode of growth, any
treatment given earlier, distant metastasis etc. were
recorded on the day of presentation. Distant
metastasis was confirmed by palpating the lymph
nodes and lymphatic channels, chest radiography
and ultrasonography of abdominal viscera.
Preliminary diagnosis was made by evaluating the
fine needle aspiration biopsy (FNAB) of the
tumorous mass. Based on the treatment regimen
followed, the animals were divided into two groups:
Group I (n= 29) in which only surgery (simple
mastectomy, enblock dissection and unilateral
mastectomy) was performed and Group II (n= 6) in
which surgery (enblock dissection) as well as
chemotherapy was done. Five animals were not
given any treatment. In all cases blood samples were
collected preoperatively, postoperatively and after
chemotherapy at required intervals for estimation
of haematological (Hb, TLC, DLC and PCV) and
biochemical (BUN, CRTN, AST, ALT, ALKP and
TP) parameters. All 35 animals of group I and group
II were kept off feed for 12 hours and off water for 6
hours prior to surgery and were premedicated with
glycopyrrolate 0.01mg/kg b.wt, butorphanol 0.2mg/
kg b.wt. and acepromazine 0.05mg/kg b.wt. mixture.
Anaesthesia was induced with 5% thiopentone
sodium and maintained with 1-2% halothane in
oxygen using Boyles apparatus. Postoperatively Inj.
Megaclox 10mg/kg b.wt. for seven days and Inj.
Melonex 0.2mg/kg b.wt. for three days were given.
In Group II at least three cycles of chemotherapy were
given starting 15 days postoperatively and each cycle
comprised of Inj. Vincristine sulphate @ 0.5mg/m²
BSA and Inj. Methotrexate @ 9-15mg/m² BSA, I/V
on day 1 followed by Tab. Cyclophosphamide @
50mg/m² BSA P.O. from 2nd to 5th day. All animals
of this group were given syrup Geriforte 1 tsf b.i.d
P.O. and syrup Alkacip @ 0.5 to 1gm (total dose)
throughout the period of chemotherapy. They were
assessed regularly for any sign of toxicity because of
chemotherapy drugs and were classified as: No or
self limiting toxicity (no apparent signs of toxicity),
mild toxicity (which could be managed by
symptomatic treatment) and severe toxicity (which
resulted in death of animal). The survival rate, mean
survival time and reoccurrence of mammary tumor
of both the groups were compared to evaluate the
effectiveness of treatment regimen.

RESULTS AND DISCUSSION
In the present study all the animals affected
with CMNs were females with median age of 10.8
years (2-15 years). Highest incidence (37.5%) was
recorded in the age group of 10-12 years whereas
the lowest incidence (2.5%) was seen in the age
group of 2-4 years and no case was reported in age
group of 0-2 years. Among the affected breeds,
the highest number of cases (57.5%) were recorded in
white spitz breed, followed by non descript breed
(15%), Doberman and crossbreed (7.5% each),
German shepherded, Cocker spaniel, Daschund and
Boxer (2.5% each). However, there seems to be a
little correlation between CMNs and breed of dog as
the population of breeds may considerably influence
the prevalence of CMNs (Singh et al. 1998). Number
of affected gland varied from one (12.5%) to ten
(15%) and in most of the cases two glands were
affected (25%). According to Harvey and Gilbertson
(1977), occurrence of multiple primary tumors in
various stages of development and of different
histologic type has led investigators to use the term
“Multicentricity of origin”; as descriptive of this
disease. Most commonly affected glands were fifth
pair (87.5%), followed by fourth pair (67.5%), third
pair (50%), second pair (37.5%) and first pair
(32.5%). Similarly, Ettinger and Fieldman (1995)
reported that approximately two third of CMN cases
occurred in the fourth and fifth pair. Majority (80%)
of the cases reported had slow growing tumors and
remaining 20% had fast growing tumors.

Out of 40 CMN cases 28 had expansive
growth pattern, 5 had infiltrative pattern, whereas 7
had both types of growth patterns. 80% of animals
with infiltrative growth pattern died during the follow up period. 10% cases of the present study were of recurrence of tumor after initial surgical removal suggesting that surgery alone was not an optimal treatment modality for CMNs. Majority of the cases did not show any nipple deformity or teat discharge but few cases showed cystic (25%) or bloody (5%) discharge. Thoracic radiograph in 26 showed soft tissue density in lung area indicating pulmonary metastasis. In 9 animals nodular pattern was visible, 5 showed milliary pattern, 3 alveolar pattern, 2 doughnut pattern, whereas mixed interstitial and bronchial pattern was evident in 16 animals. Tiemessen (1989) reported that the sensitivity, specificity and accuracy of thoracic radiography for detection of lung metastasis were 65%, 97% and 87% respectively. No metastatic lesion was visible in abdominal viscera on ultrasonography. Ultrasonography (n= 23) of tumors revealed that 56.52% (13) were non-cystic, 21.74% (5) were cystic and rest 21.74% (5) were of mixed nature. In 13 cases, tumor showed ill defined borders and attachment with the underlying tissue; of which 8 died in the follow up period. Nyman et al. (2005) reported that irregular borders, invasiveness of tumorous growth, high vascularity and heterogenous echotexture were indicative of malignant growth. Out of 35 cases in which FNAB was performed 27 were diagnosed accurately from cytology indicating 77.14% diagnostic accuracy of cytology. The sensitivity, specificity, positive and negative predictive values of cytology were 78.78%, 100%, 100% and 12.5% respectively. Ghilseni (2006) reported 89.3% sensitivity, 97% specificity, 99.4% positive predictive value and 68.7% negative predictive value of cytology in relation to histopathology. Histopathology of 35 cases revealed that 33 were neoplastic (32 malignant and 1 benign) and 2 were non neoplastic (1inflammation and 1 chronic mastitis with hyperplasia). All the 35 animals recovered uneventfully except in two cases healing was delayed for 15 days due to seroma formation.

In group I no significant difference was seen in the mean survival time after surgery among different excisional techniques. Similarly, Misdrop and Hart (1979) and Palta (2000) reported that animals treated by enblock dissection and mastectomy had similar post surgical survivability. At the time of presentation, the mean values of TLC and DLC were within the normal physiological range, however, the mean PCV and Hb values were less than normal range in both group I and group II. A non significant decrease in Hb and PCV values and increase in TLC at time of presentation was also reported by Bala (2005). No significant change in any of the haematological parameter was recorded in group II during the course of chemotherapy, suggesting minimum haematological toxicity of the chemotherapy regimen used. This was probably due to the fact that the nadir usually occurs 5-7 days following treatment and neutrophil count returns to normal within 36-72 hours. However, a non significant decrease in TLC value was seen after first cycle of therapy although the value was within normal range. Todorova et al. (2005) reported significant decrease in leucocytes count after every cycle of doxorubicin and cyclophosphamide therapy when compared to the values before surgery.

The mean serum values of AST and ALT were within normal physiological range before and after surgery in both the groups (Tables 1 and 2). In the animals of group II there was a non significant increase in AST (38.50 ± 7.314 IU/L to 58 ± 8.012 IU/L) and ALT (30.667 ± 6.367 IU/L to 46.60 ± 6.974 IU/L) serum values after second cycle of therapy which might be due to increase in metabolic activity of liver for detoxification of the drugs (Benzamin 1979 and Todorova et al. 2005). In contrast, Palta (2000) reported no change in AST and ALT values after neoadjuvant and adjuvant

<table>
<thead>
<tr>
<th>TABLE 1: Biochemical values (mean ± SE) in group-I before and after surgery</th>
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<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>AST (IU/L)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
</tr>
<tr>
<td>CRTN (mg/dL)</td>
</tr>
<tr>
<td>TP (g/dL)</td>
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<tr>
<td>ALKP (IU/L)</td>
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</table>
chemotherapy with vincristine in CMN cases. There was no change in mean values of BUN and CRTN in both the groups I and II before and after surgery. However in group II, there was a mild increase in BUN after first cycle and creatinine values after 1st and 2nd cycles of therapy but the values were within normal physiological range (Table 1). Riley and Riley (1982) noted a progressive increase in BUN throughout the period of chemotherapy with vincristine, cyclophosphamide and methotrexate. The mean serum ALKP values were towards the higher limit of normal range in both the groups at the time of presentation. There was a significant difference (p< 0.05) in preoperative and post operative serum values of ALKP in both groups I. Many workers (Chopra and Saxena 1997, Gill 1997, Nayyar 2002, Bala 2005 and Sharon 2007) reported elevated levels of ALKP at presentation of CMN cases. There was a progressive increase in serum ALKP values after first and second cycle of therapy (Table 2). A significant increase (p<0.05) was seen in the value of total protein in group I from preoperative 5.875 ± 0.3568g/dL to 6.485 ± 0.330g/dL following surgery. In group II there was no significant change in total protein values during the course of therapy but it remained towards the higher side of normal range. Other workers (Riley and Riley 1982, Sandhu 1995, Palta 2000 and Todorova 2005) also reported the similar findings in their studies.

Out of 29 animals of group I, 13 died, 3 did not report back and 13 were leading disease free life till the end of study. The survival rate was 50% with mean and median survival time of 169.33 ± 4.99 days and 158 (15-365) days respectively. The median age of live verses dead animals were 10.5 years and 10 years respectively. The median age of live verses dead animals were 10.5 years and 10 years respectively. Similarly, Sandhu (1995) reported that following surgery 61% of CMN cases died within one year. According to Simon et al. (1996), 49.3% of the cases showed tumor recurrence and/or metastasis (mainly pulmonary) within one year of surgery.

In group II out of 6 animals 3 died in the follow up period and three were alive without any recurrence till the end of study. On the basis of TNM staging system 4 animals had poor prognosis of which two died and remaining 2 had good prognosis of which one died of ancylostoma infestation after second cycle of therapy. The survival rate was 50% with mean and median survival time as 162 ± 19.60 days and 137.5 (55-365) days respectively. The live and dead animal median age was 7.5 (6-9) years and 12 (6-12) years respectively. Very few signs of toxicity were shown by the animals for 4-5 days after therapy which included anorexia, restlessness, nausea, vomiting etc. and no major complication was seen during the course of therapy. Das et al. (1991) studied the efficacy of vincristine, methotrexate and cyclophosphamide alone or in combination in the treatment of canine transmissible venereal sarcoma (CTVS) and found complete and sustained remission of tumors with no side effects.

There was no significant difference in mean survival time and survival rates of group I and group II. This was in contrast with findings of many other workers (Theilen and Madewell 1979, Ravikumar et al. 1999 and Jain 2006) who reported that chemotherapy along with surgery was better than surgery alone. The significant finding in the present study was that all the live animals in group II were of less than 8 years of age, suggesting that the present combination is safe and effective in comparatively young animals. Though patients may be benefitted from the use of a single chemotherapeutic agent; more often it is advantageous to use multiple drugs in combination. Chemotherapy is currently the method of choice for adjuvant therapy of advanced disseminated neoplasia, since the primary surgical treatment or radiotherapy can be less readily applied after metastasis has occurred; and immunotherapy is not suited to destroy the large tumor masses (Theilen and Madewell 1979). From the present study

### TABLE 2: Biochemical values (mean ± SE) in group-II before surgery and before every chemotherapy cycle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Surgery</th>
<th>Before First Cycle</th>
<th>Before Second Cycle</th>
<th>Before Third Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>38.50 ± 7.31</td>
<td>45.714 ± 6.772</td>
<td>38.833 ± 7.314</td>
<td>58.00 ± 8.012</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>25.00 ± 6.367</td>
<td>34.571 ± 5.894</td>
<td>30.667 ± 6.367</td>
<td>46.60 ± 6.974</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>9.400 ± 2.544</td>
<td>7.857 ± 2.355</td>
<td>13.217 ± 2.544</td>
<td>10.34 ± 2.787</td>
</tr>
<tr>
<td>CRTN (mg/dL)</td>
<td>0.892 ± 0.159</td>
<td>0.880 ± 0.148</td>
<td>1.240 ± 0.159</td>
<td>1.160 ± 0.175</td>
</tr>
<tr>
<td>TP (g/dL)</td>
<td>6.533 ± 0.705</td>
<td>7.329 ± 0.653</td>
<td>6.117 ± 0.705</td>
<td>6.580 ± 0.772</td>
</tr>
<tr>
<td>ALKP (IU/L)</td>
<td>93.667± 11.994</td>
<td>38.571± 11.105</td>
<td>65.667 ± 11.994</td>
<td>84.40 ± 13.139</td>
</tr>
</tbody>
</table>
it is concluded that FNAB is a reliable method to give tumor diagnosis preoperatively and must be used routinely for making early diagnosis. Majority of CMNs are malignant and if early diagnosis can be made in comparatively young animals the combination of surgery and chemotherapy used can prolong the survival time without showing much signs of toxicity.

REFERENCES


