



*Original Research*

## Pre-Operative Cytology- An Effective Tool in the Diagnosis of Malignant Canine Mammary Tumours

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### Abstract

The present study was conducted on 24 cases of canine mammary tumour (CMT) to evaluate cytology versus histopathology in its diagnosis. During surgical intervention samples were taken for cytology and histopathology by using FNAB and excisional biopsy respectively. The tumours were also sub-classified and graded on the basis of histopathology. On the basis of cytology all the tumours were found to be malignant which was confirmed by histopathology. A comparison of cytology and histopathology revealed a cent-percent positive correlation in detecting malignancy and tumour sub-type. However, there was poor correlation between cytology and histopathology in predicting the grade and stage of malignancy.

**Key words:** CMT, Correlation, Cytology, Grade, Histopathology, Stage

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### Introduction

Mammary gland tumors are the most common neoplasms in dog after the skin tumours. These tumours constitute approximately 50% of all tumors in female dogs, and 82% of the female reproductive tract. According to previous studies about 50% (Moe, 2001) of the mammary gland tumours are malignant, but according to most studies in India almost 90% (Gupta *et al.*, 2012; Sharma, 2017) of these tumours are malignant which may be due to ignorance or late reporting by the owner. Therefore, there is a need of diagnostic techniques which can preoperatively determine the nature of CMT, so that appropriate treatment could be established (Hellmen *et al.*, 1993). Cytological examination has been found to be quick and reliable



technique for the diagnosis of mammary tumours. The present study was conducted to determine the comparative efficacy of cytology vis-a-vis histopathology in the diagnosis of CMT.

### Materials and Methods

The study was conducted on 24 dogs suffering from mammary gland tumours presented to the Small Animal Clinics of the Department of Teaching Veterinary Clinical Complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, during the period from November 2017 to July 2018. The study was approved by Institutional Animal Ethics Committee vide application no. GADVASU/2017/IAEC/42/4. Clinical and physical examination of dogs having mammary tumours were done. During surgical intervention the samples were taken for histopathology in 10 % neutral buffered formalin and cytology using FNAB and excisional biopsy respectively. The cytological samples were stained with Leishman stain (Jain, 2006). Classification of tumours was done on the basis of the behavior of tumour (Tyler *et al.*, 1999) and on the basis of tissue of origin i.e. histogenic classification (Alleman and Bain, 2000). The criteria of malignancy were as following: anisocytosis, pleomorphism, hypercellularity, macrokaryosis, anisokaryosis, multinucleation, increased nuclear/cytoplasm ratio; nuclear molding, increased mitotic figures; abnormal mitoses; coarse chromatin pattern and anisonucleosis. The tumors were classified as malignant when three or more above mentioned criteria were present in the cytologic smears. The formalin fixed tissues were processed for paraffin sectioning and stained by H&E technique (Bancroft and Gamble, 2008). The tumours were graded as per Goldschmidt *et al.*, 2011, whereas, the osteochondrosarcoma were graded as per Kirpensteijn *et al.*, 2002. Both cytological and histopathological diagnosis were done by two pathologists separately and then correlated.

Sensitivity, specificity and diagnostic efficacy of cytology vis-à-vis histopathology was determined by the method given by Gertsman and Capucci. 1986. In addition Kappa correlation coefficient and Fisher Exact test was used for determining the correlation and association between cytologic and histopathologic findings respectively using Statistical Package for Social Sciences (SPSS) for windows version 20.0 (IBM SPSS INC, Chicago, Illinois).

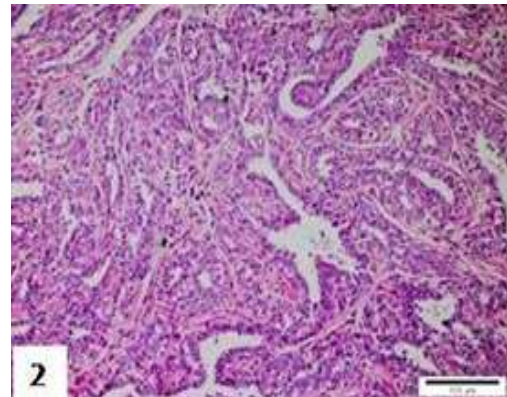
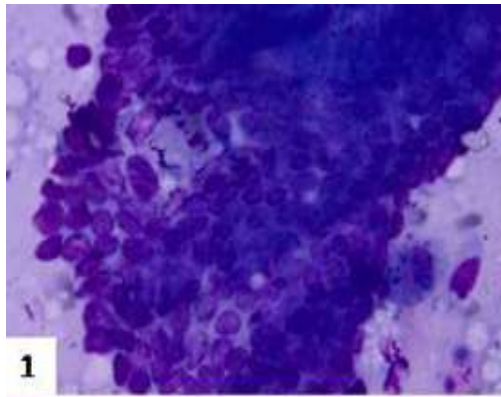
### Results and Discussion

On the basis of cytological examination 22 cases were found to be malignant and two were inconclusive due poor retrieval of cells. Out of 22 cases, 15 cases were classified as carcinosarcoma (62.5%) (Figs.1&7) and 7 (29.16%) (Figs.3, 5&9) as carcinoma. Histopathological examination was done in all 24 cases. On histopathological examination, all the tumours were found to be malignant, which were in agreement with cytological diagnosis. On the basis of histological examination, 11 cases each were diagnosed as carcinoma (45.83%) and carcinosarcoma (45.83%) (Fig. 8) and two cases were diagnosed as sarcoma (8.34%)(Table

1). The carcinomas were further classified as early carcinoma, simple tubular carcinoma, tubulopapillary carcinoma (Fig. 2), adenosquamous carcinoma (Fig. 4), solid carcinoma (Fig. 6), anaplastic carcinoma and complex carcinoma. Both the cases of sarcoma were classified as osteochondrosarcoma (Fig.10).

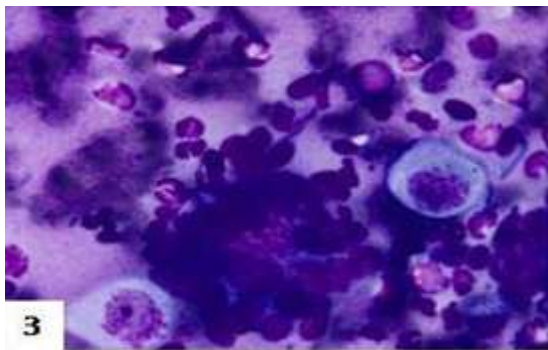
**Table 1:** Comparison of cytological and histopathological diagnosis

Cytological diagnosis	Histopathological diagnosis
Carcinoma ( n= 7)	Early carcinoma (1)
	Adenosquamous carcinoma (1)
	Tubulopapillary carcinoma (1)
	Anaplastic carcinoma (1)
	Simple tubular carcinoma (1)
	Simple tubular carcinoma (1)
	Solid carcinoma (1)
Carcinosarcoma ( n= 15)	Carcinosarcoma (9)
	Tubulopapillary carcinoma (2)
	Solid carcinoma ( 2)
	Complex carcinoma (1)
	Osteochondrosarcoma (1)
In-conclusive (2)	Osteochondrosarcoma (1)
	Carcinosarcoma (1)

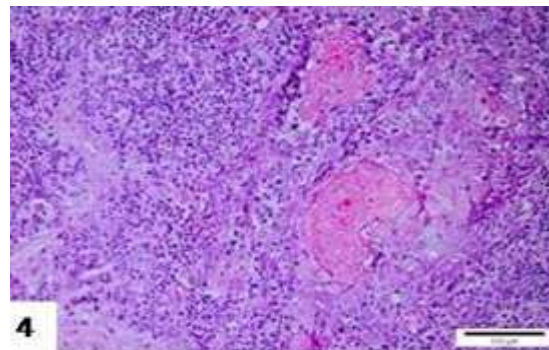


**Fig. 1:** FNAB smear of carcinosarcoma depicting cluster of neoplastic cells embedded in bluish ground substance. (Microscopically diagnosed as tubulapappillarycarcinoma. Fig.2). Leishman stain, Original magnification1000 X.

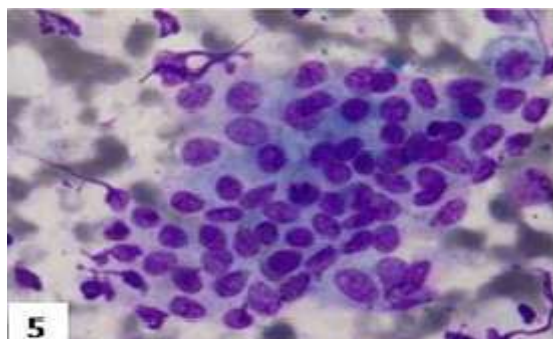
**Fig. 2:** Microphotograph of tubulopapillary carcinoma showing cells arranged in tubular pattern along with papillary projections. (Cytologically diagnosed as carcinosarcoma. Fig. 1). H&E, Original magnification 100 X.



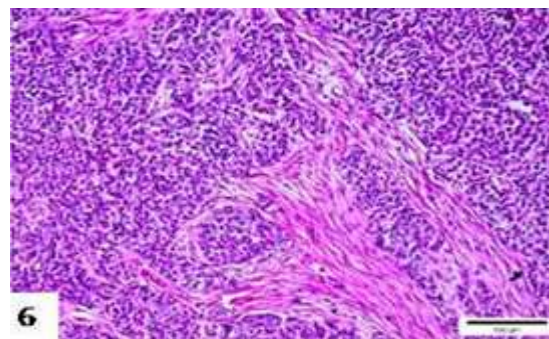
**Fig. 3:** FNAB smear of carcinoma showing neoplastic epithelial cells along with squamous differentiation and many neutrophils indicating inflammation. (Microscopically diagnosed as adenosquamous carcinoma. (Fig. 4). Leishman stain, Original magnification 1000 X.



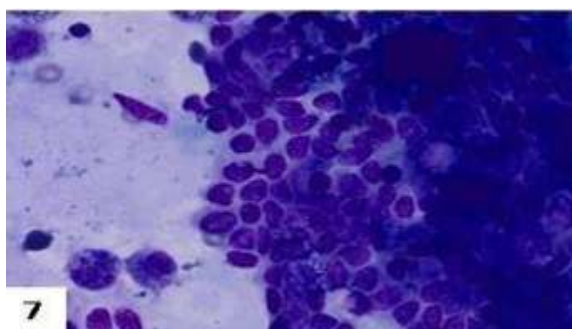
**Fig. 4:** Microphotograph of solid carcinoma showing adenosquamous differentiation in places. (Cytologically diagnosed as inflammatory carcinoma. Fig. 3). H&E, Original magnification 200X.



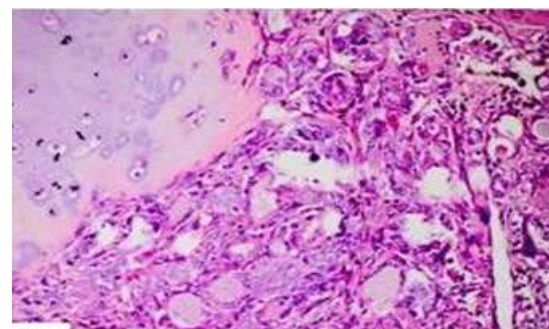
**Fig. 5:** FNAB from a case of carcinoma showing slightly pleomorphic epithelial cells arranged in clusters. (Microscopically diagnosed as solid carcinoma. Fig.6). Leishman stain, Original magnification 1000 X.



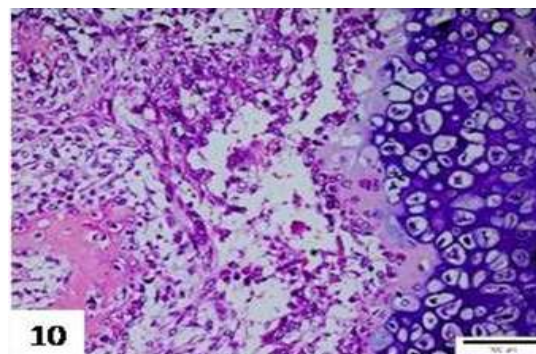
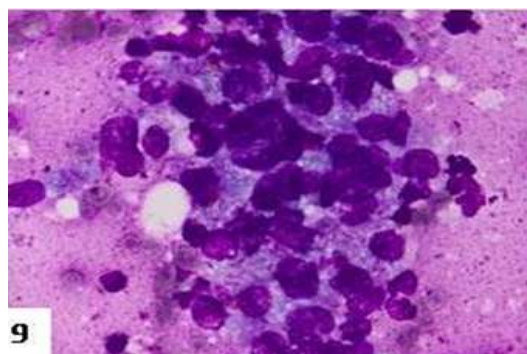
**Fig. 6:** Microphotograph of solid carcinoma showing cells arranged in form of sheet with lot of connective tissue indicating schirrous pattern. (Cytologically diagnosed as carcinoma. Fig.5). H&E, Original magnification 100x.



**Fig. 7:** FNAB smear from a case of carcinosarcoma showing mixed population of epithelial and connective tissue cells and moderate amount of purplish blue colored ground substance depicting cluster of neoplastic cells embedded in bluish ground substance. (Microscopically diagnosed as carcinosarcoma. Fig. 8). Leishman stain, Original magnification 1000 X.



**Fig. 8:** Microphotograph of carcinosarcoma showing clear cut differentiation of epithelial and connective tissue components. (Cytologically diagnosed as carcinosarcoma. Fig. 7). H&E, Original magnification 200 X.



**Fig. 9:** FNAB from a case of carcinoma showing slightly pleomorphic epithelial cells arranged in clusters. (Microscopically diagnosed as chondrosteosarcoma. Fig.10). Leishman stain. Original magnification 1000 X

**Fig.10:** Microphotograph of osteochondrosarcoma showing chondromatous differentiation. (Cytologically diagnosed as carcinoma. Fig.9). H&E, Original magnification 200 X

All the tumours in the present study on the basis of cytology and histopathology were diagnosed as malignant, which may be due to ignorance by the owner or late detection of the tumour (Gupta *et al.*, 2012; Jain, 2006; Kishor *et al.*, 2016). On the contrary other workers reported low incidence of malignancy in CMT. (Sorenmo *et al.*, 2009). The most common type of tumor on the basis of cytology was carcinosarcoma – 68.18% (15 cases), followed by carcinoma (31.81%) (7 cases). The findings were similar to Sangha *et al.* (2011), who reported a higher incidence of carcinosarcoma on cytological evaluation. On the contrary, Ezerskytė *et al.* (2011) reported a relatively higher incidence of carcinomas in their study. On the basis of histopathology, incidence of carcinomas and carcinosarcomas was observed equally, which is in accordance with the findings of other workers (Ezerskytė *et al.*, 2011; Nayyar, 2002). In contrast, (Gupta *et al.*, 2012) reported a higher incidence of carcinosarcoma as compared to carcinoma in canines.

### Correlation between Cytological and Histopathological Diagnosis

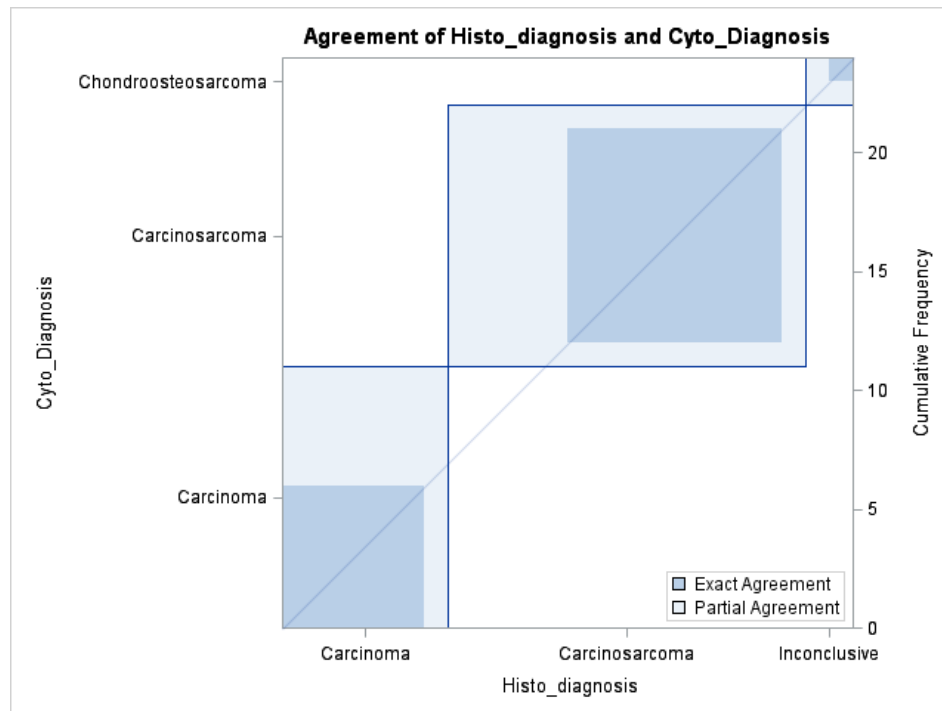
Cytology was done in 24 cases, out of which 22 were diagnosed as malignant and two were inconclusive because of poor retrieval of cells. All the 22 cases were diagnosed as malignant on the basis of cytology which were further confirmed as malignant on the basis of histopathology. Thereby the cytology was 100% effective in detecting the malignancy of tumour. The results from the present study were in agreement with the previous studies (Simon *et al.*, 2009; Cassali *et al.*, 2007 and Sangha *et al.*, 2011). In the present study, a statistically significant correlation was observed between the histopathological and cytological diagnosis (Table 2). On the basis of Fisher's Exact test a statistically significant association (P-value 0.0323) was also observed between the histopathological and cytological findings (Fig.11). As per method given by Gertsman and Capucci 1986, a sensitivity of 88% and the correlation of 62.55% was observed between cytological and histopathological diagnosis. However, in the present study, on the basis of Fisher's Exact

test, no significant association was observed between grade of the tumour and histopathological sub-typing as well as between grade and the stage of the tumour.

**Table: 2** Correlation between cytological and histopathological diagnoses of CMT

Simple Kappa Coefficient	
Kappa	0.4182
95% Lower Conf Limit	0.1033
95% Lower Conf Limit	0.733
Test of H0: Kappa = 0	
Z	2.6395
One-sided P > Z	0.0042
Two-sided P >  Z	0.0083

Similar to our study high sensitivity and specificity for cytological and histopathological diagnosis was observed by previous workers (Simon *et al.*, 2009 and Sangha *et al.*, 2011). Human breast cancer studies also corroborated the above findings (Choi *et al.*, 2004; Saravanja *et al.*, 2005).



**Fig. 11:** Depicting agreement between cytological and histopathological diagnoses of CMT

### Conclusion

The present study concluded that cytology is as effective method in diagnosis of malignant CMT and a statistically significant positive association was observed between cytological and histopathological sub-typing of the CMT.

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