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Specificity and Sensitivity of Cytological Techniques for Rapid Diagnosis of Neoplastic and Non-neoplastic Lesions of Canine Mammary Gland

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Abstract

The present study aimed at evaluating diagnostic accuracy and efficacy of cytology for rapid diagnosis and differentiation of various mammary affections of canines and the comparison of cytological findings with that of histopathology. Cytological evaluation was conducted on 33 dog patients having mammary gland lesions. Out of these, 28 cases were diagnosed for various mammary affections and rest five cases were inconclusive on the basis of cytological evaluation. Among various affections, 23 were diagnosed as tumors, four as mastitis and one as a case of steatitis. Comparison of cytological findings with histological diagnoses yielded 70% concordance between the two. For malignant mammary tumours, the correlation was 92.30% while for benign mammary tumours, it was quite low (66.66%). Among malignant tumours (n=13), there was one false positive case (7.69%) and one false negative case (7.69%). One case of mixed mammary tumour was misdiagnosed as simple adenocarcinoma. In benign tumours, only one false negative (3.3%) result was found and there was no false positive result. Various cytological techniques like touch impression, fine needle biopsy and teat fluid cytology were employed and these techniques yielded sensitivity and specificity of cytological diagnoses as 87.5% and 85.7%, respectively. The positive and negative predictive value of cytology for diagnosing mammary tumours was 93.3% and75%, respectively. Overall, cytology was found most accurate in diagnosis of neoplastic lesions followed by inflammatory lesions and hyperplastic lesions.

Key Words: Dog, cytology, histopathology, mammary tumours, fine needle aspiration cytology.

Introduction

Mammary gland of dogs is the most common site for development of affections like tumours, mastitis, steatitis, galactostasis, galactorrhea, agalactia etc. The affections like agalactia, galactostasis and galactorrhea are the result of abnormal physiology of the patient and respond to standard drug therapies. However, the occurrence of mammary tumour in a dog is life threatening. The canine mammary tumour (CMT) account for between 25 to 50 percent of all the tumours occurring in the canines (3,12,34) and are only second to skin tumours (31).While analyzing the incidence by site, breast cancer in women is the most frequent (32%), the first of all neoplasia (52%) occurring in bitches and the third (17%) in queens after lymphohaemopoietic and skin tumours (20,19,27). Keeping in view, the high rate of occurrence, rapid onset of growth, and poor survival statistics of the patients, a quick and early diagnosis of mammary tumour is required.

Today, the worldwide tendency is to look for quick and inexpensive method of tumor diagnosis as the routinely used histological method requires invasive sampling and long hours for processing of the tissue samples. Cytology refers to microscopic evaluation of cells. The technique was first used in the United States

by Guthrie in 1921(18). It is a quick, inexpensive, less painful and easily repeatable technique requiring minimal of sophisticated instruments. Furthermore, implantation of cancer cells after this technique is very rare (11).

Diagnostic cytology has recently been employed for diagnosis of some of the diseases in dogs and cats (9,24,28,29). When applied to mammary gland lesions, the method shows good diagnostic accuracy. More recently, valuable additional information has been provided by cytological features of fine needle aspirates or from imprints of tissue sections (33,39). In some studies, combination of cytology, radiology and clinical assessments has resulted in accurate diagnosis in 99% of cases (38).

However, contradictions have been directed towards the tendency to report accuracy rates and failure to identify the applicability of fine needle aspiration cytology in clinical veterinary practice. Similarly, the information regarding comparison of cytological diagnosis with histopathological diagnosis is very sparse (25) and contradictory in veterinary literature. Some results show 79% correlation of cytologically established diagnosis to the ultimate histological tests (21,41) while according to other studies, it is 63% or less (30). So, the present study has been focused to diagnose and differentiate canine mammary gland lesions on the basis of cytological evaluation and furthermore, to compare the relative efficacy of diagnostic cytology with histopathology.

Materials and methods

A total of 33 dogs with mammary affections were examined for cytological studies. Complete history of each case like age, sex, breed, season, duration and location of affection and clinical signs observed were recorded. The lesions were observed for consistency, teat discharge, or any ulceration etc. Cytological samples were collected either as fine needle aspirate from affected area or tissue imprints from excised tissue. Fluid cytology was also done in cases showing teat discharge. The cellular smears were categorized as neoplastic, inflammatory or hyperplastic type of lesion according to cellular and nuclear details of the smear (Table 1).

Table 1: Cellular and nuclear characteristics of cytological smears from canine mammary gland affections.

Neoplastic lesion	Inflammatory lesion	Hyperplastic lesion
Hypercellularity,overlapping of cells. Pleomorphism,anisocytosis, macrocytosis. Multinucleation. Higher nuclear to cytoplasmic ratio. Abnormal shaped nucleoli (angular, polygonal). Coarse nuclear chromatin Abnormal mitotic figures.	**Abundant inflammatory cells like neutrophils, eosinophils etc with few or no normal tissue cells.**	Small round to oval shaped condensed nuclei Relatively constant nuclear to cytoplasmic ratio **More immature type of cells**

The smears showing neoplastic lesions were further classified on the basis of tissue of origin (1) and the behavior of tumour (36). Grading of tumours was done by scoring the cellular smears as per the criteria used by Mouriquand and coworkers (32) as shown in Table 2. The cytological classification of mammary tumors as benign or malignant was done as per the criteria described by Tyler *et al.* (36).

For Histopathological studies, tissue samples were taken from the multiple sites of the tissue mass excised after surgery of the patient. After fixation in 10% neutral buffer formalin, the tissue samples were processed and the sections were stained with Mayer's-hematoxylin and eosin using standard procedure (26). The classification of mammary gland tumours was done as per WHO Histological Classification of Mammary Tumours of the Dog and Cat (7). Finally, the diagnostic value of cytology in diagnosing mammary gland affections of canine was calculated in the terms of sensitivity, specificity and predictive values (13). The correlation between cytological and histological diagnosis was also determined.

Table 2 : Cytoprognostic classification (32)

	Morphology	Score
Cells	Isotated	3
	In clusters	0
	Large size	3
Nuclei	Anisokaryosis	2
	Naked	3
	Budding	2
	Hypochromasia	3
	Hyperchromasia	2
Enlarged Nucleoli	Red	3
	Blue	2
Mitosis	>3/slide	1
	>6/slide	3

Grade was obtained by adding the different scores obtained;
Grade I : Score <5
Grade II : Score 5-9
Grade III : Score >10
When a double malignant cell population was found in same tumour, the most anaplastic cells were taken in to account for scoring purpose.

Technique for collecting cytological specimen:

To collect fine needle aspirate, the effected mammary gland was held firmly in position with free hand and the puncturing site was cleaned with alcohol. A 22 gauze needle attached with 10 ml syringe was inserted in to the mass. A strong negative pressure was applied by withdrawing the plunger to about 3/4th of the volume of the syringe. The needle was moved through the mass three or four times in different directions. Still with the needle in mass, suction was slowly released. The needle was then removed from the mass and the syringe from the needle. The syringe was then filled with little air, reconnected to the needle, and the contents of the needle blown on to one or more clean dry slides, which were rapidly air dried. This procedure was repeated from multiple sites of effected gland.

For making tissue imprints, the tissue excised after the surgery was cut to get a fresh uncontaminated surface. The cut surface was blotted many times to make it dry. A clean glass slide was pressed against the dried tissue surface and the cellular smears were spread either as starfish or squash preparations. Staining of smears was done with different Romanowsky stains like Wright stain, Leishman stain, Wright- leishman combination etc. using standard procedures (10).

Results

From 33 cases examined cytologically, 69.69% cases were diagnosed as neoplastic lesions, 15.15 % as inflammatory lesions (12.12% as mastitis, 3.03% as steatitis) and 15.15% cases were inconclusive. As per tissue of origin, cytological smears of neoplastic lesions were classified as epithelial, mesenchymal or mixed type. Twelve mammary tumours were found to be epithelial in origin, 2 (8.69%) of mesenchymal and 9 (39.13%) were of mixed type. The finding was consistent with the work of other researchers (40) who reported that tumours of epithelial origin dominate among mammary tumours of dogs. Adenocarcinomas dominate the epithelial type of tumours (47.82%), whereas adenomas constitute only 4.34%.

Cytologically, fine needle aspirates of adenocarcinomas were characterized by anisocytosis, macrocytosis, hypercellularity, multinucleation, increased nuclear to cytoplasmic ratio and prominent nucleoli (Figure 1). Histologically, these tissue sections were comprised of cancerous epithelial cells characterized by hyperchromasia, enlarged nuclei, prominent nucleoli and increased mitotic figures replacing normal acini of mammary gland (Figure 2). Fine needle aspirates of inflammatory adenocarcinoma revealed isolated or clusters of hyperchromatic epithelial cells with enlarged nuclei, prominent nucleoli and coarse chromatin. Inflammatory cells like neutrophils and lymphocytes infiltrate the surrounding tissue (Figure 3). Histologically, the tissue sections revealed same picture as of adenocarcinoma but in addition there was marked infilteration of lymphomononuclear cells in the acini (Figure 4).

In contrast to adenocarcinomas, fine needle aspirates of adenomas yield clusters of morphologically uniform population of well differentiated cells (Figure 5). Histologically, adenomas were comprised of well differentiated epithelial cells replacing normal acini of mammary gland along with focal areas of calcification in some cases of mixed mammary adenoma (Figure 6).Tissue sections of papillary adenoma were characterized by papillary projections in lumen of acini and those of cystic adenoma revealed majority of acini containing mammary secretions with marked proliferation of epithelial cells lining the acini.

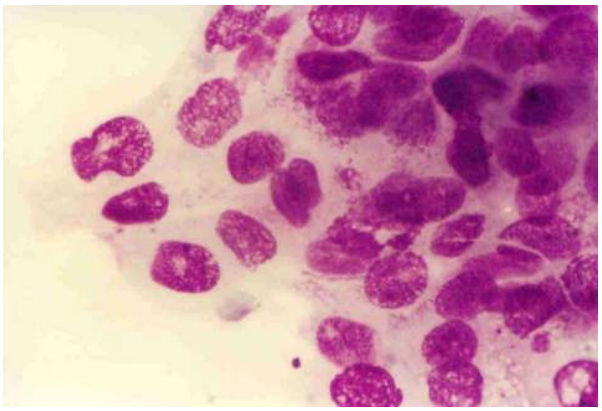

Figure 1. Adenocarcinoma: Fine needle aspirate with hypercellular pleomorphic, large hyperchromatic naked cells with coarse chromatin and prominent nucleoli, Wright x 750.

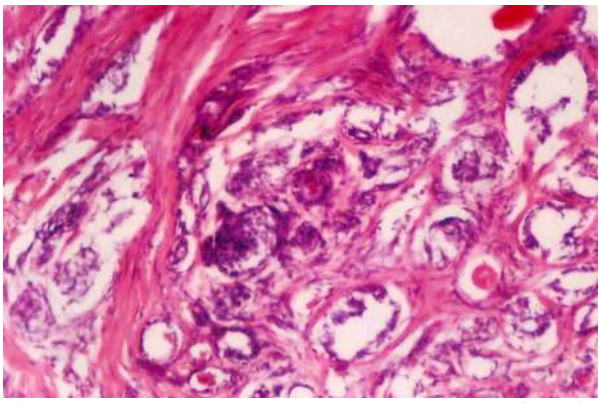

Figure 2. Adenocarcinoma: Composed of acini separated by fibrous tissue septa. Cancerous epithelial cells of acini exihibit hyperchromasia and hypercellularity, H&E x 75.

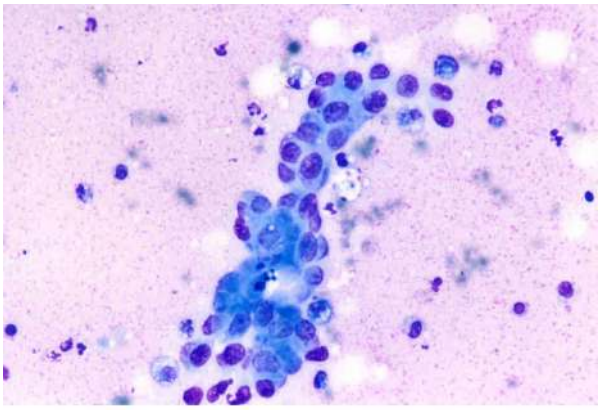

Figure 3. Inflammatory adenocarcinoma: Touch impression consisting of hyperchromatic cells with enlarged nuclei having prominent nucleoli, more basophilic cytoplasm and intracytoplasmic vacuoles. Inflammatory cells infilterate the surrounding area, Wright-Leishman x 150.

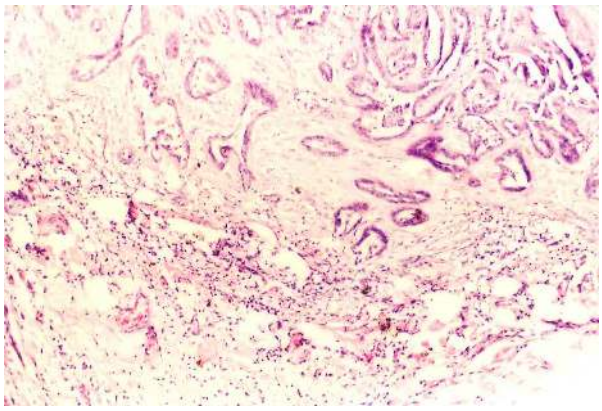

Figure 4. Inflammatory adenocarcinoma: Adenocarcinomatous cells replacing the acini beside being marginated by inflammatory cell infilteration, H&E x 150.

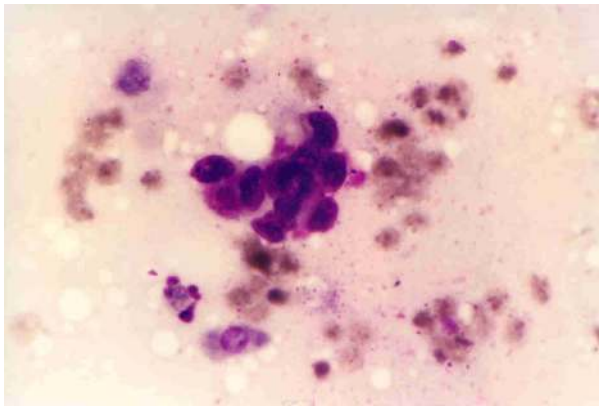

Figure 5. Adenoma: Fine needle aspirate composed of clusters of clusters of cells with highly basophilic cytoplasm, variable shaped nuclei and increased nuclear to cytoplasmic ratio, Wright-Leishman x 300.

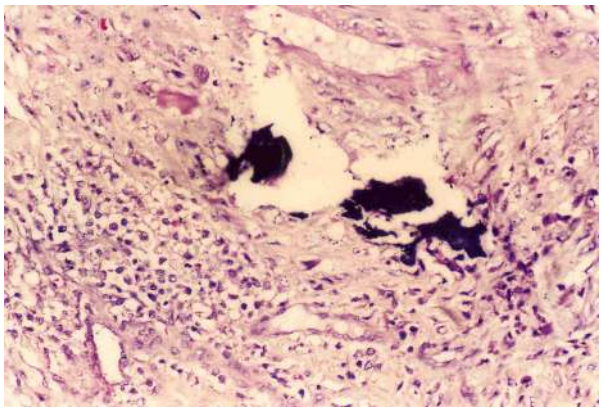

Figure 6. Mixed mammary adenoma: Consist of mixture of epithelial (adenoma), myoepithelial and mesenchymal cells. A combination of acini, fat cells, fibrous tissue alongwith calcification is present, H&E x 150.

In mixed type of origin, mixed mammary adenocarcinomas were dominant type (34.78%) and mixed mammary adenoma contribute to 4.34% only. Cytologically, the aspirates of mixed mammary adenocarcinomas were hypercellular containing hyperchromatic cells with enlarged round, oval or

stellate shaped nuclei, prominent macronucleoli and coarse chromatin (Figure 7). Histopathologically, these were found to be comprised of adenocarcinoma, fibroma and osteoid component. The adenocarcinoma component was characterized by formation of adenomatous structure formed by neoplastic glandular epithelium, fibroma contained stellate shaped fibroblasts and osteoid component contained bony tissue (Figure 8).

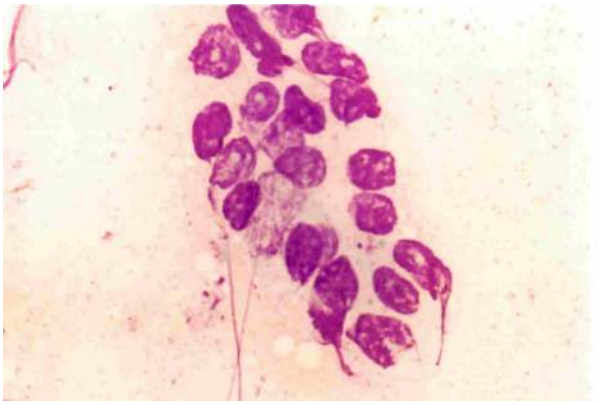

Figure 7. Mixed mammary tumor: Fine need aspirate containing oval to spindle shaped pleomorphic cells, Wright-Leishman x 750.

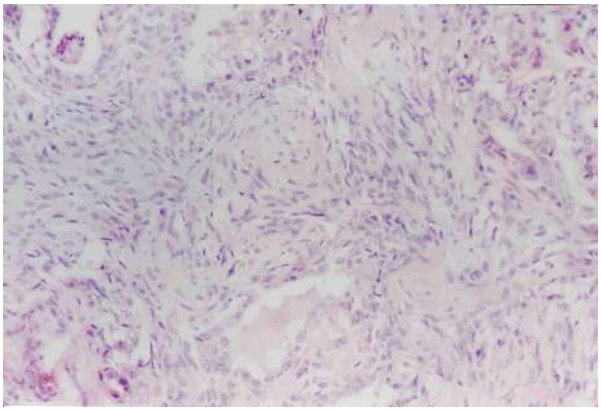

Figure 8. Adenocarcinoma: Composed of acini separated by fibrous tissue septa. Cancerous epithelial cells of acini exihibit hyperchromasia and hypercellularity, H&E x 75.

In tumours with mesenchymal origin, both liposarcoma and lipoma were diagnosed (4.34% each). Fine needle aspirate of liposarcoma was characterized by hypercellularity, pleomorphism, anisokaryosis, cells with vacuolated cytoplasm and nuclei displaced to periphery of cells (Figure 9). These cells were containing abundant cytoplasm histologically (Figure 10), but the cell membrane was difficult to appreciate cytologically. Small to large fat vacuoles were observed in background of smear.

One case of lipoma was diagnosed in a 3 year old female German shepherd on cytological basis. Smears made from fine needle aspirate of lipoma were oily and did not dry in air. Cytologically, smears revealed single or group of large fat cells containing large volume of pale cytoplasm and outlined by a single thin membrane. The nucleus was small, dense and pushed to periphery of cell (Figure 11).

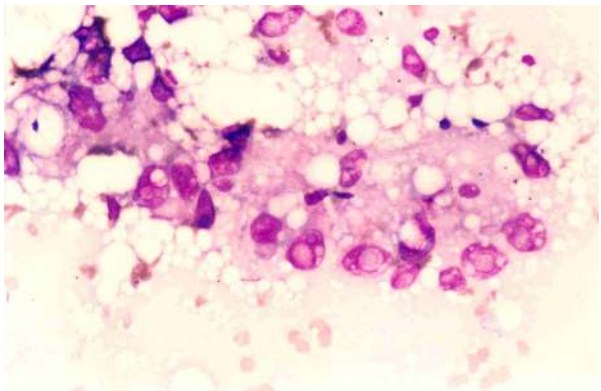

Figure 9. Liposarcoma: Very cellular fine needle aspirate smear composed of spindle to oval shaped cells with highly basophilic cytoplasm containing with intracytoplasmic lipid vacuoles displacing nuclei to periphery of cells, Wright x 150.

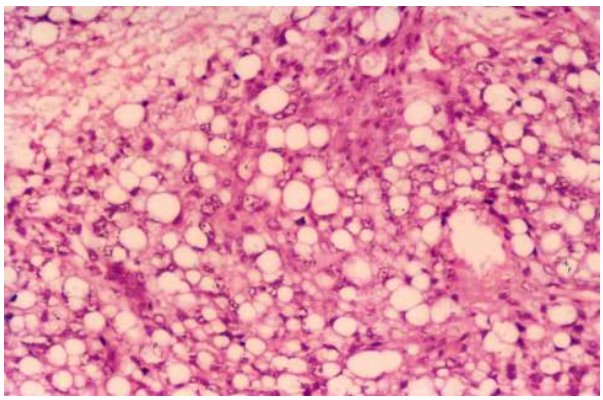

Figure 10. Liposarcoma: Characterized by variable sized mature fat cells arranged in sheets and have eccentric nuclei, H&E x 75.

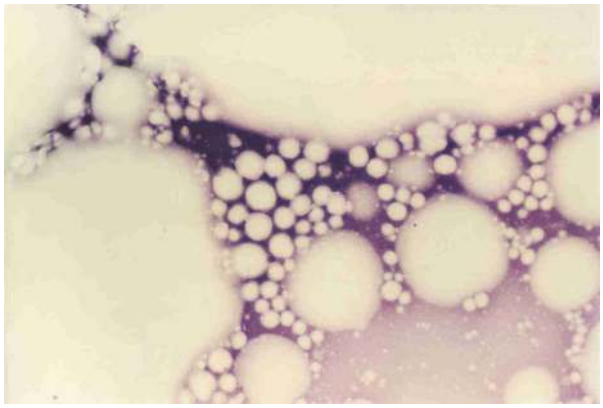

Figure 11. Lipoma: Poorly cellular fine needle aspirate smear with abundant free lipid residue from disrupted adipocytes, Wright x 150.

In cases of inflammatory lesions, cytologic examination of secretions from inflammed mammary glands revealed bacteria and numerous degenerative neutrophils (Figure 12). Samples of milk taken from affected mammary gland of bitches formed dense gel

on addition of sodium lauryl sulphate reagent. Furthermore, colour change of milk drop from yellow to green was observed an addition of bromothymol blue reagent to it. Both these tests confirmed the presence of high degree of mastitis in affected animals.

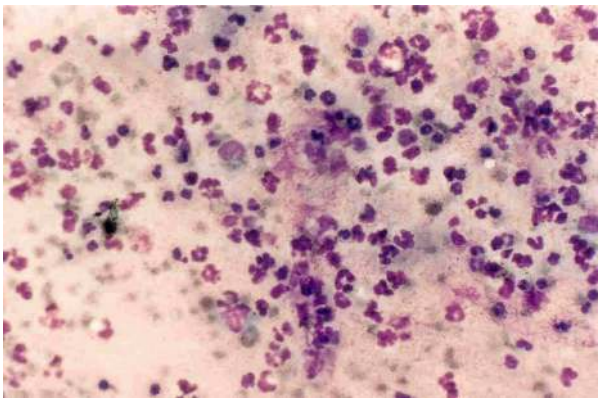

Figure 12. Mastitis: Fine needle aspirate showing numerous degenerative neutrophils and bacteria, Wright-Leishman x 300.

One case of steatitis was observed in a 12 year old Pomerian. The affected teats were hard, swollen and yellowish in colour. The area around cranial teat was ulcerated and pus was oozing out of it. Cytologically, the aspirate revealed inflammatory cells (neutrophils, monocytes) infiltrating the adipose tissue (Figure 13).

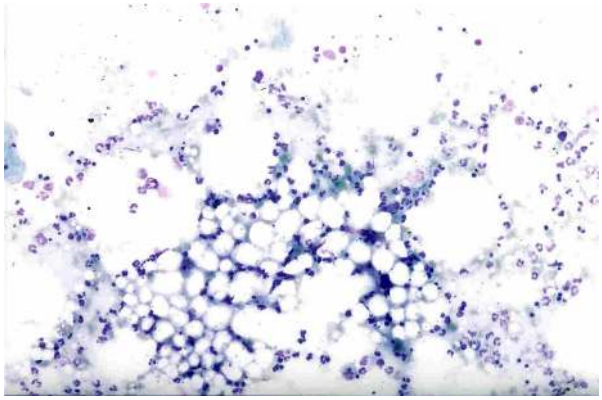

Figure 13. Steatitis: Fine needle aspirate consisting of inflammatory cells infilterating the adipocytes, Wright-Leishman x 150.

Classifying neoplastic lesions as benign or malignant according to criteria of malignancy used by Tyler *et al* (36), three cases (13.04%) were found to be benign while twenty (86.96%) were found to be malignant (Table 3). Malignant type of mammary tumors dominantly contributed to 86.96% with adenocarcinomas and mixed mammary adenocarcinomas constituting to 40% each, followed by inflammatory adenocarcinoma as 15% and liposarcoma 5%. Among the benign type of mammary tumours, one case each of adenoma, mixed mammary adenoma and lipoma was diagnosed. Cytoprognostic classification of cytological smears following scoring system (32) revealed none of the malignant tumours to be of Grade I, four of Grade II and sixteen of Grade III.

Table 3: Cytological Diagnosis of neoplastic affections of canine mammary gland.

Tumour	No. of cases	%age
Malignant	**20**	**86.96**
Adenocarcinoma	8	40
Mixed mammary adenocarcinoma	8	40
Inflammatory adenocarcinoma	3	15
Liposarcoma	1	5
Benign	**3**	**13.04**
Adenoma	1	33.33
Mixed mammary adenoma	1	33.33
Lipoma	1	33.33

Comparison of Histopathological studies with cytological findings:

Out of 33 cases which were diagnosed on cytology basis, tissue sections for histopathological examination were available for 20 cases only. For the rest of cases, either the owner did not turned up for surgery or the owner was advised not to get animal operated considering metastasis of tumour to visceral organs or debilitating conditions of aged animal. Out of these 20 cases, 14 cases (70%) were in complete accordance with cytological diagnosis (Table 4). On correlating cytological findings with histological diagnosis (Table 5), there were three false positive (15%) and two false negative (10%) results. Among the 13 malignant tumours, there was one false positive case (7.69%) and one false negative case (7.69%). One case of mixed mammary tumour was misdiagnosed as simple adenocarcinoma. Only one false negative (3.3%) result was found in benign tumours and there was no false positive result. In diagnosing neoplasia, cytology had a sensitivity of 87.5% and specificity of 85.7%, calculated as described by Gerstman and Cappucci (13). The positive and negative predictive values of cytology for diagnosing tumours were 93.3% and 75%, respectively.

Table 4 **:** Comparison of Cytological findings with histological diagnosis.

Cytological Diagnosis	No. of cases	Histological Diagnosis
Mixed mammary Adenoma	1	Mixed mammary Adenoma
Adenoma	1	Papillary adenoma
Lipoma	1	Mastitis
Adenocarcinoma with inflammatory cells (AIC)	2	Ductular AIC
	1	Mixed mammary adenocarcinoma
Adenocarcinoma	2	Adenocarcinoma
	1	Hyperplasia with chronic inflammation
Mixed mammary adenocarcinoma	6	Mixed mammary adenocarcinoma
Liposarcoma	1	Liposarcoma
Mastitis	1	Mastitis
Inconclusive	1	Cystic adenoma
	1	Mixed mammary adenocarcinoma
	1	Mastitis

Table 5: Correlation between Cytological and Histological diagnoses of canine mammary gland affections.

Type of lesion	Number of cases	False positive		False negative		Correlation
A. Neoplastic	n	n	%	n	%	%
Benign tumours						
Cystic adenoma	1	-	-	1	33.33	0
Papillary adenoma	1	-	-	-	-	100
Mixed mammary adenoma	1	-	-	-	-	100
Total	3	-	-	1	33.33	66.66
Malignant tumours						
Adenocarcinoma	2	-	-	-	-	100
Mixed mammary adenocarcinoma	8	1	7.69	1	7.69	75
Adenocarcinoma with inflammatory cells	2	-	-	-	-	100
Liposarcoma	1	-	-	-	-	100
Total	13	1	7.69	1	7.69	92.30
B.Inflammatory (Mastitis)	3	1	33.33	1	33.33	33.33
C. Hyperplasatic	1	1	100	-	-	0
Total	20	3	15	3	15	70

Discussion

The results of present study revealed that by evaluating cellular and nuclear details of a cellular smear, it is possible to diagnose the nature of lesion; whether neoplastic, inflammatory or hyperplastic type. This can help the physician to make a preliminary idea about the mode of treatment within a few seconds after the sample had been taken from the patient who is presented first time to the hospital. Further, it was also possible to determine the origin of tumour as cytological smears from the mammary tumours of epithelial origin were characterized by cells usually situated in clusters along with round to oval nuclei and clear cytoplasmic boundaries. In contrast to this, mesenchymal tumours consisted of individually settled polygonal/ elliptical/ stellate cells with poorly defined cell membrane. Mixed type of tumours revealed cellular elements of both epithelial and mesenchymal type. In addition to this, we were also able to detect the nature of tumour (benign or malignant) from a cytological smear as per the criteria of malignancy (36). The smears from malignant tumours were highly anisocytic, hypercellular and contain larger number of pleomorphic cells with increased and abnormal mitotic figures as compared to cellular smears of benign tumours. Above all these, it was also possible to detect the severity of the tumour by grading it on the basis of scores given to nuclear and cellular parameters of the smear (32). This provides an ultimate and immediate clue for the

prognosis of disease and helps the physician to plan the line of treatment well in time. Thus, cytology is the first hand tool available to the physician in making a quick diagnosis.

In diagnosing neoplasia, cytology had a sensitivity of 87.5% and specificity of 85.7%, calculated as described by Gerstman and Cappucci (13). The positive and negative predictive values of cytology for diagnosing tumours were 93.3% and 75%, respectively. The findings were comparable to those reported by Ghilseni *et al* (14) and Chalita *et al* (15), who reported 89.3% and 89% sensitivity, respectively with 99.4% and 100% positive predictive values, and 68.7% and 96% negative predictive values, respectively. However, specificity obtained in present study (85.7%) was different from studies of Ghilseni *et al* (14) and Chalita *et al* (15), who reported specificity to be 97.4% and 100%, respectively. It's reason could be variability in sample collection or individual skill. In present study, correlation between cytological and histopathological diagnosis for malignant tumours was above 90 percent while it was quite low (66.66%) for benign tumours (Table 5). The results were at variance with findings of Simeonov and Stoikov (35), who reported 84.6% and 80.70% of correlation between cytological and histological diagnoses of benign and malignant tumours. In previous studies on canine mammary lesions, this agreement ranged from 25% (2) to 45% (17). These values are low when compared with results on studies of human breast lesions which described 93.1% (8) to 96.7% (5) agreement between cytological and histopathological diagnosis of mammary tumours, respectively. The results from the present research showed a higher percentage of false positive and false negative diagnoses than results of other authors (21,30,35,41). The reason could be low quality staining techniques (6,8,16) contamination of smear by blood or other body fluids or cellular atypia caused by simultaneously occurring reactive inflammatory processes (4,37).

Among different cell retrieval techniques, tissue imprints (touch impression) were found to be more diagnostic than fine needle aspirates. The reason could be the fact that while making impression, the wider area of affected tissue came in contact with slide and thus enhanced obtaining of cellular material. Moreover, the specimen could be easily collected from exact site of affected tissue (visible grossly) many a times without any difficulty like sudden movement of animal, bending of needle in hard tissue etc. However, obtaining of cellular material by means of fine needle aspiration combined with simultaneous preparation of tissue imprints was recommended. This opinion was supported by other authors as well (9,22,35). Furthermore, Long *et al* (24) reported higher accuracy with touch and smear preparations, compared with medium pressure impressions in the cytologic diagnosis of intracranial lesions. Teat fluids were also found to be diagnostic for cancer lesions in some of cases (9.09%). So among different cell retrieval techniques touch impression techniques was found to be best followed by fine needle biopsy and teat discharge smears.

In spite of expanding true positive cases of cytological testing, the diagnostic value of this method has many limitations. One of the major imperfections was "geographic miss" i.e. puncturing the lesion from inappropriate site and thus leading to misdiagnosis so multiple aspirates were needed from multiple site of lesion in order to improve diagnostic value. The entry of blood in syringe while taking aspirate or entry of tissue fluid on pressing slide hard over the excised tissue while making touch impression contributed to contamination of smear and thus diminished diagnostic accuracy of cytology. Further, differential diagnosis was very difficult in cytologic preparations of hyperplastic lesions and adenoma and histological examination was required for accurate characterization. However, as already shown by other studies in veterinary medicine, fine needle aspiration cytology had an edge over histopathological examination in terms of providing immediate diagnosis and thus timely determination of plan of action (23). But the detailed classification of mammary gland lesions required histological testing as well.

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