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Fig. 1: Squamous cell carcinoma. Ether: ethanol fixed FNAB smear showing cluster of neoplastic cells, anisokaryosis and prominent nucleoli. MGG x 800

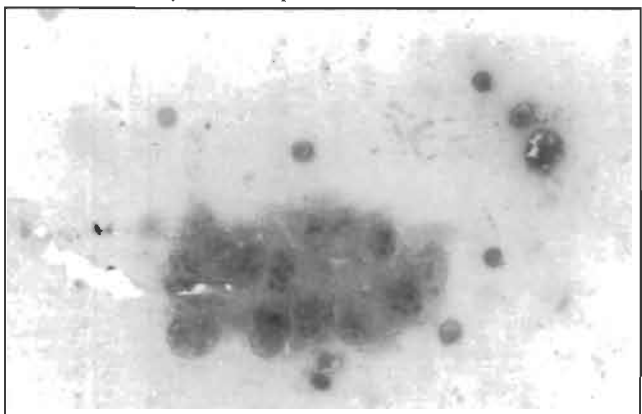


Fig. 2: Cystic papillary adenoma. Ether: ethanol fixed impression smear showing roughly spherical to cuboidal neoplastic cells arranged in a glandular pattern. WG x800.

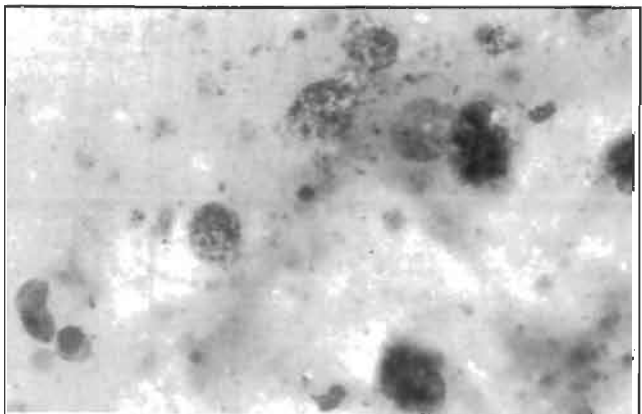


Fig. 3: Malignant mixed mammary gland tumour. Ether: ethanol fixed impression smear showing neoplastic cells with multi vacuolated and granulated basophilic cytoplasm. WG x 800

Cystic papillary adenoma of mammary gland:

Ether:ethanol fixed WG stained FNAB and impression smears revealed high cellularity, acinar pattern, cluster of oval shaped cells, anisocytosis and anisokaryosis, basally placed nuclei, increased nuclear: cytoplasmic ratio, multinucleated cells and reticular chromatin (Fig. 2). Impression and FNAB smears of MGG and Diff-Quik stained specimens yielded low cellularity that affected the interpretations.

Malignant mixed mammary tumour:

Ether:ethanol fixed FNAB and impression smears stained with WG revealed moderate cellularity, cluster of cuboidal cells, multivacuolated granulated basophilic cytoplasm (Fig. 3), Anisocytosis, anisokaryosis, eccentric nuclei, increased nuclear:cytoplasmic ratio and coarse chromatin. Distinct nucleoli were observed in Wright's stained smear. Diff-Quik stained ethanol fixed FNAB smears revealed anisokaryosis and cytoplasmic granules. The MGG stained smears revealed neoplastic cells with eccentric nuclei (signet ring cells), foamy cytoplasm and basophilic granules. Similarly canine mammary tumours have been identified with cytological features⁹.

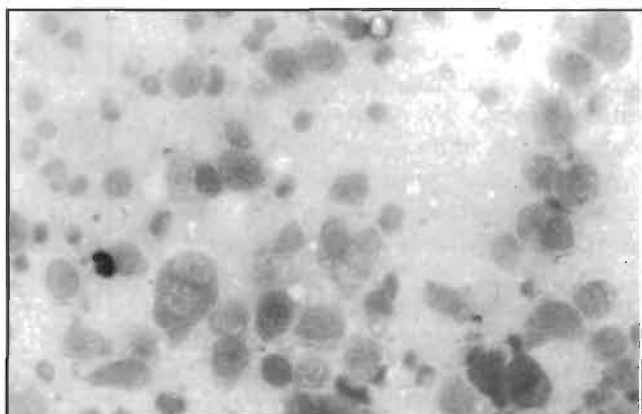


Fig. 4: Osteosarcoma. Methanol fixed impression smear showing neoplastic cells with multiple nucleoli. WG x 800.

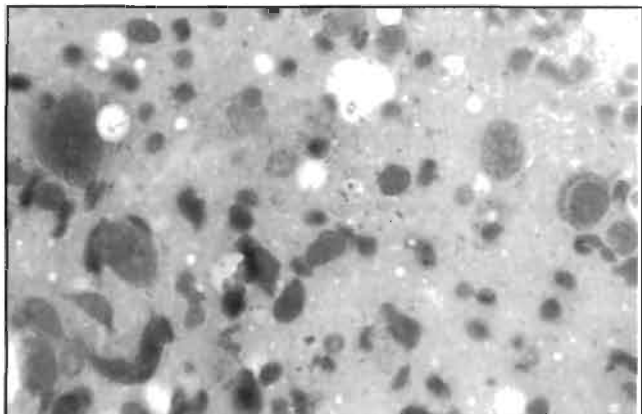


Fig. 5: Osteosarcoma. Methanol fixed impression smear showing fusiform (1), round (2) and plasmacytoid cells with perinuclear clear zone (3) and RBCs. WG x 800.

CYTOLOGICAL AND HISTOPATHOLOGICAL DIAGNOSIS OF CANINE SKIN TUMOURS

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ABSTRACT

Nine canine skin tumours were studied by cytology and confirmed by histopathology. Fine needle aspiration biopsy (FNAB) technique was found to be superior to impression and teased smear techniques as it yielded high cellularity in epithelial tumours. However, impression smears were best suited for mesenchymal tumours. Ethanol and ether: ethanol (1:1) fixatives were better than air drying and methanol fixations. Cytoplasm stained well with May-Grunwald Giemsa in osteosarcoma and haemangiopericytoma. Nucleoli stained well with Wright's stain in osteosarcoma. Diff-Quik was suitable for immediate diagnosis. Characteristic cytological features like plasmacytoid appearance with perinuclear space of Golgi zone (osteosarcoma), swirling pattern of tadpole shaped cells (haemangiopericytoma,) and signet ring cells (mixed mammary gland tumour) were seen. Cytological diagnosis yielded definite results in epithelial tumours than in mesenchymal tumours barring osteosarcoma.

Key words: Canine, cytology, fixation, FNAB, skin tumours, staining

INTRODUCTION

In canines, skin and subcutis were the commonest sites of occurrence of tumours as this accounted for 67.5 per cent of the total neoplasms³. An attempt was made to develop and standardise cytological techniques in quick diagnosis of canine cutaneous tumours.

MATERIALS AND METHODS

Samples were collected from nine suspected cases of malignancy in dogs that were brought for surgical interventions to Department of Clinics, Madras Veterinary College, Chennai-600 007. Fine needle aspiration biopsy (FNAB) was performed on all cases⁶. Impression smears were prepared from excisional biopsies. The smears were air dried and wet fixed with 95% ethanol, ether:ethanol (1:1) or methanol. The FNAB and impression smears were stained with Wright's, Wright-Giemsa (WG), May-Grunwald-Giemsa (MGG) and Diff-Quik¹. For histopathology pieces of tissues from the excised tumours were fixed in 10 per cent formol saline, embedded in paraffin and sectioned to stain with haematoxylin and eosin (HandE).

RESULTS AND DISCUSSION

Data pertaining to age, breed, sex and gross lesions were recorded (Table 1). Haematological study (Table 2) indicated anaemia in the 2nd, 4th and 9th cases (Hb-7-8 g/dl, PCV- 16-28 %, RBC- 3.24-4.62 m/cmm) leucocytosis with absolute neutrophilia and lymphocytosis in 5th, 6th and 9th cases and leucocytosis with absolute neutrophilia in the 1st and 7th cases. All the cases were subjected to

histopathological examination and were diagnosed as tumours. Their cytological observations are discussed.

Ether:ethanol and 95% ethanol fixation yielded better results than with air-dried and methanol fixations. Cell distortions were found in methanol fixations. The FNAB smears yielded high cellularity in epithelial tumours whereas impression smears yielded high cellularity in mesenchymal tumours².

Squamous cell carcinoma: The FNAB of ether : ethanol fixed MGG stained smear revealed cell clusters. The cells were angular and contained basophilic cytoplasm, large nuclei and showed increased nucleus nucleolar ratio and more than one nucleoli (Fig. 1).

Similar observations were recorded earlier^{4,8} which also included variations in the nuclear:cytoplasmic ratio, ghostly appearance of nuclei⁴ and finely stippled, moderately clumped chromatin⁸.

Sweat gland adenocarcinoma: The FNAB smears fixed in ether:ethanol and stained with WG revealed moderate cellularity, cluster of cells with indistinct vacuolated cytoplasmic outline, anisocytosis and anisokaryosis, increased nuclear:cytoplasmic ratio and oval or round nuclei with fine chromatin and multiple prominent nucleoli. Ether:ethanol fixed impression smears stained with Wright's, MGG and WG stains yielded low cellularity which intervened in interpretations. Similar citations were also made by others⁴. They also reported hyperchromatism, voluminous nucleoli, nuclear basophilia and vacuolization of cytoplasm.

Ceruminous gland adenocarcinoma: The WG stained ether:ethanol fixed impression smears revealed cluster of cuboidal cells, anisocytosis, anisokaryosis, abundant granulated cytoplasm with indistinct outline

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and darkly stained basally located basophilic nuclei and increased nuclear:cytoplasmic ratio. The MGG stained ethanol fixed impression smears revealed hazy indistinct nuclei. These findings concurred with the observations of others^{4,5}. Goldschmidt and Schofer⁵ also observed irregular eccentric nuclei, high mitotic index and large droplets of secretory materials at apical pole.

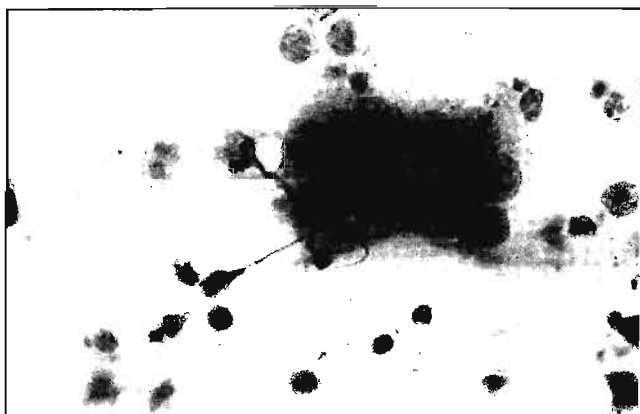


Fig. 1: Squamous cell carcinoma. Ether: ethanol fixed FNAB smear showing cluster of neoplastic cells, anisokaryosis and prominent nucleoli. MGG x 800

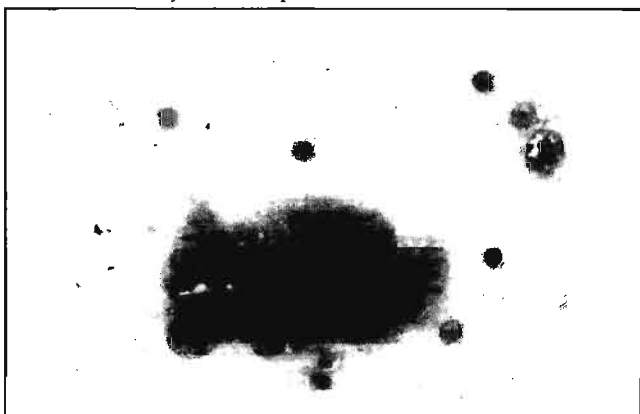


Fig. 2: Cystic papillary adenoma. Ether: ethanol fixed impression smear showing roughly spherical to cuboidal neoplastic cells arranged in a glandular pattern. WG x800.

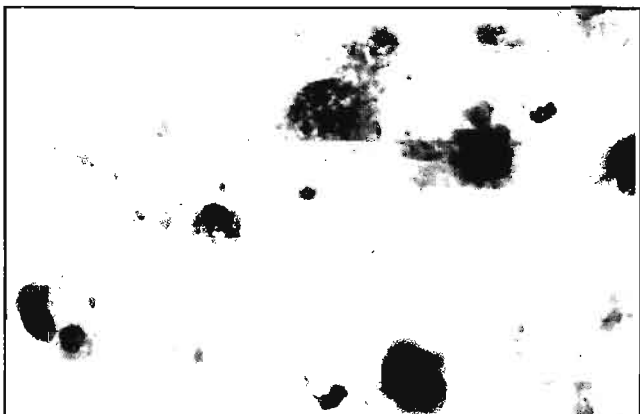


Fig. 3: Malignant mixed mammary gland tumour. Ether: ethanol fixed impression smear showing neoplastic cells with multi vacuolated and granulated basophilic cytoplasm. WG x 800

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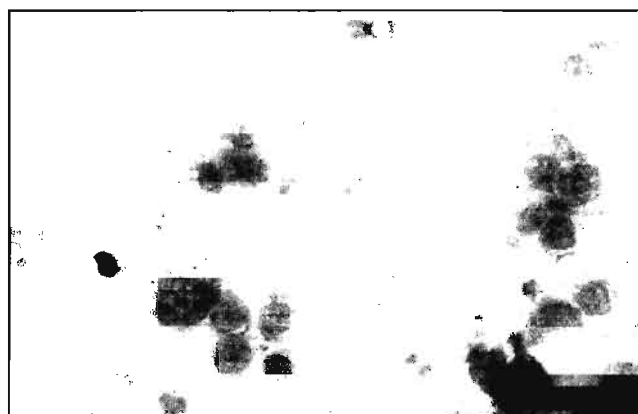


Fig. 4: Osteosarcoma. Methanol fixed impression smear showing neoplastic cells with multiple nucleoli. WG x 800.

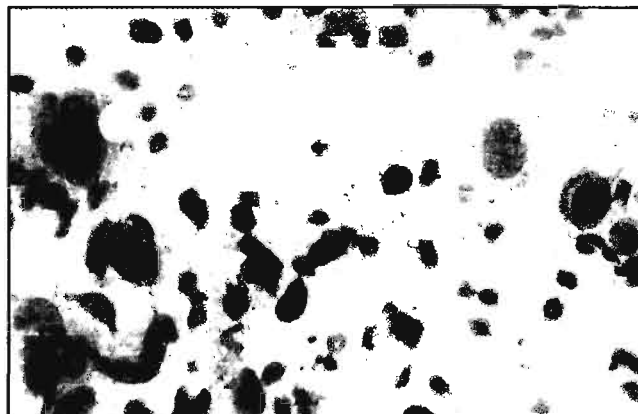


Fig. 5: Osteosarcoma. Methanol fixed impression smear showing fusiform (1), round (2) and plasmacytoid cells with perinuclear clear zone (3) and RBCs. WG x 800.

Table 1: Animal particulars, gross morphology and microscopic diagnosis of canine tumours studied

Case No.	Age (Years)	Sex	Breed	Location	Size (dia)	Shape	Weight (g)	Colour and appearance	Cut surface	Consistency	Histopathological Diagnosis
1	7	F	Mongrel	Mammary gland and perineum	10cm	Round	80-100	Dark gray with skin intact	Fetid smelling cauliflower like greyish growth	Soft pulpy	Squamous cell carcinoma
2	7	F	Mongrel	Brisket and ventral thorax	15 cm	Oval multi nodular	500-800	Reddish brown with fetid ulcerated foul smelling discharge	Grayish tan	Firm	Sweat gland adenocarcinoma
3	10	F	German Shepherd	Base of the left ear	5 cm	Conical	50	Gray and skin intact	-	Firm	Ceruminous gland adenocarcinoma
4	11	F	Mongrel	Mammary gland	10cm	Irregular	250	Reddish brown ulcerated and necrosed	Spongy red	Firm	Cystic papillary adenoma
5	17	F	Mongrel	Mammary gland	11cm	Cauliflower like growth	250	Reddish	Whitish grayish lobulated and cartilagenous	Firm	Malignant mixed mammary tumor
6	7	F	Spitz	Vagina	5 cm	Ovoid	100	Yellowish brown	White and glistening	Firm	Fibroleiomyoma
7	7	F	Spitz	Nasal bridge	10 cm	Irregular	100	Greyish white	Slimy, rusty and gray colored	Firm and tough	Chondroma
8	6	M	Doberman	Left shoulder region				Not done			Osteosarcoma
9	12	F	Mongrel	Left fore limb	10 cm	Round	10	Gray	Greyish pink	Firm	Hemangiopericytoma

M - Male, F - Female

Fibroleiomyoma: Ether:ethanol fixed FNAB smears stained with Wright's, WG, Diff-Quik and MGG showed fusiform cells, eosinophilic cytoplasm and basophilic nuclei with centrally placed nucleoli. Wright's, MGG and Diff-Quik stain yielded very low amount of cells that made cytological interpretation difficult.

Chondroma: The WG stained ether:ethanol fixed impression smears revealed basophilic cytoplasm, oval or spherical nuclei with distinct nucleoli and coarse chromatin. Ether:ethanol fixed MGG stained impression smears revealed the cells embedded in the basoeosinophilic cartilagenous matrix substance. Cytologically, basoeosinophilic cartilagenous matrix substance containing large binucleated cells with basophilic cytoplasm were observed. Cytoplasm contained azurophilic granules. These findings concurred with earlier report⁴.

Osteosarcoma: Diff-Quik stained smears revealed deeply basophilic cytoplasm with azurophilic granules. The WG and MGG stained smears revealed foamy cytoplasm and multiple nucleoli. Impression smears of osteosarcoma yielded less cellularity and cells were fusiform to round shape, which matched with the previous work⁷. Cytological features of osteosarcomas such as deeply basophilic cytoplasm with azurophilic granules, Golgi zone and plasmacytoid appearances were observed (Fig. 4.5). These findings agreed with those of Fleury *et al.*⁴.

Haemangiopericytoma: Ether:ethanol fixed FNAB and impressions stained with WG revealed moderate cellularity, cell clusters, anisocytosis, anisokaryosis and increased nuclear:cytoplasmic ratio. Diff-Quik stained smears revealed swirling pattern of spindle cells with centrally placed nuclei (tadpole cells). Wright's and WG stained smears revealed centrally placed bulged nuclei. The MGG stained smears revealed one or two distinct nucleoli and stippled reticulated chromatin. These concurred with earlier findings^{4,8}. Among them the latter authors also observed indistinct or wispy cytoplasmic borders.

Cytological technique is quick, simple and inexpensive for tumour diagnosis⁶. The FNAB yielded high cellularity than impression smear for epithelial tumours. Ethanol (95%) and ether:ethanol were found to be better fixatives than methanol and air-dried fixations. The FNAB smears stained with WG showed better nuclear and cytoplasmic details. Diff-quick staining can be preferred for immediate diagnosis.

Table 2: Haemogram, radiography and histopathological diagnosis of canine cases studied

No.	Hb (g/dl)	ESR (mm/1h)	PCV (%)	Total Count		Differential Count (%)					Radiographic details		Histopathological diagnosis
				RBC (millions/c u.mm)	WBC (thousand/ cu.Mm)	N	L	M	E	B	View	Result	
1	10	20	28	4.52	17	70	11	3	10	-	-	-	Squamous cell carcinoma
2	7	14	20	3.24	10.4	80	15	4	1	-	Thorax	No metastatic lesion	Sweat gland Adeno carcinoma
3	12	27	34	5.82	14	84	12	3	1	-	Thorax	No metastatic lesion	Cervious gland adenocarcinoma
4	7	14	28	3.84	14.9	68	22	5	5	-	Thorax	Pulmonary infiltration	Cystic papillary adenoma
5	10	20	35	5.15	48	88	10	2	-	-	Thorax	Pulmonary infiltration	Malignant mixed mammary tumor
6	9.5	19	29	4.72	40	81	17	1	1	-	Abdomen	No uterine involvement	Fibro-leiomyoma
7	12	24	30	4.5	19	72	10	5	1	-	-	-	Chondroma osteosarcoma

