Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 5 Issue 9 September 2023

Research Article

Comparative Analysis of Cytological and Histological Grading Techniques in Canine Mammary Carcinomas

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DOI: 10.31080/ASVS.2023.05.0749

Received: July 20, 2023

Published: August 29, 2023

Abstract

The study was conducted in samples of mammary carcinomas obtained from dogs that were brought to University Veterinary Hospitals, Mannuthy and Kokkalai during a study period of one year. The study employed cytological grading system proposed by Robinson., *et al*. (1994) in human breast carcinomas (HBCs) with necessary modifications. Histopathological grading was done according to the inputs by Clemente., et al. (2010) which is a modification of the Nottingham system of malignancy grading in HBCs. The grades obtained for carcinomas in both the evaluations were compared and absolute concordance rate as well as separate concordance rates for each grade of carcinoma was assessed. The results revealed satisfactory concordance (87.5 per cent) and a significant positive correlation between the two methods of grading mammary carcinomas in canines. Further, the sensitivity and specificity of cytological grading was assessed with respect to histological grading and the results revealed that cytological grading was 100 per cent specific in case of grade III carcinomas and 100 per cent sensitive for grade I carcinomas. The study concluded that cytological evaluation of fine needle aspirate from canine mammary carcinomas can give the clinician firsthand information regarding the selection of treatment methods to be adopted before surgical resection.

Keywords: Canine Mammary Carcinomas; Cytology; Concordance; Grading

Introduction

Examination of smears made from fine needle aspirates of mammary tumours in canines is a less invasive and comparatively easy technique that gives a preliminary insight regarding the aggressiveness of the tumour. Assessment of various morphological features of the cells and nuclei present in the stained smears help the clinician to predict the nature of the tumour. Benign and malignant lesions can be clearly differentiated and the mode of treatment can be planned accordingly. Robinson., *et al*. (1994) described a system for cytological grading of HBCs based on six different cytological parameters namely the extent of cell dissociation, cell uniformity, cell size, nature of nuclear margin, appearance of nuclear chromatin and presence/absence of nucleolus. This cytological grading system corresponded satisfactorily with well-established histological grading systems and hence, became the widely recommended system for cytological diagnosis of HBCs. In the current study, Robinson's system of grading formed the basis of cytological evaluation of smears made from fine needle aspirate of canine mammary tumours (CMTs). Since CMTs are often associated with increased inflammatory and necrotic changes when compared to HBCs, certain pertinent modifications envisaged for CMTs, were also taken into consideration while evaluating the cytological smears.

Materials and Methods

The samples were collected from 50 dogs suspected for mammary tumours that were presented to Kerala Veterinary and Animal Sciences University hospitals at Mannuthy and Kokkalai during a period of six months. Cysts and abscesses were excluded from the study.

Collection of aspirate and preparation of cytology smears

A 22-gauge needle attached to a 5 ml syringe was used to collect aspirates. Aspirates were collected from different areas of the tumour mass. A minimum of five smears were prepared from each case and stained with Field's stain. Only high-quality smears with adequate staining and preserved cell morphology were considered for evaluation.

Cytological evaluation

For cytological evaluation, along with the six diagnostic criteria proposed by Robinson for HBCs, additional features like presence of inflammatory cells including foamy macrophages, naked nuclei, RBCs, background debris, mucosecretory material and syncytia formation were also evaluated for grading the samples. The samples were initially classified as malignant, benign and hyperplastic. The malignant carcinomas were further subjected to cytological grading as per the revised score card proposed for cytological grading by recent researchers [9]. The score card incorporated Robinson's criteria along with the previously described features.

Histopathological evaluation

Representative samples of tumour tissues were collected in neutral buffered formalin and processed for histopathological examination. Tissue sections of 4-5 µm thickness were prepared, stained by routine haematoxylin and eosin method, mounted with DPX and examined.

The tissue sections were analysed according to the system proposed by Clemente., *et al.* (2010), and histological malignancy grading was done by considering three main parameters namely tubule formation, nuclear pleomorphism and mitotic counts. Individual scores under each of these categories were summed up to arrive at the final score and grades were assigned accordingly.

Evaluation of concordance between two grading systems

The cytological and histological grades were compared and absolute concordance rate as well as separate concordance rate for each cytological grade of tumour was assessed with respect to the histological grades obtained. The sensitivity and specificity of cytological grading with respect to histological grading was also calculated.

Results

Among the 50 samples examined, after excluding the cysts, abscesses, benign and hyperplastic lesions, 24 were suggestive of epithelial type malignant tumours namely carcinomas and hence, comparative analysis between cytological and histological grading was done employing these samples. Cytological grade 1 carcinomas comprised cluster of smaller monomorphic cells with uniform nuclear pattern and indistinct nucleoli. Inflammatory cells, RBCs and necrotic material were only minimal in all the examined fields. There was no appreciable syncytia formation or mitotic figures in grade 1 carcinomas (Figure 1). Nine cases were observed as grade 1 carcinomas. Grade 2 carcinomas consisted of cells both in dissociated form as well as in clusters. The cells were comparatively bigger and showed moderate pleomorphism, syncytia formation and mitotic figures could be appreciated in low levels. Nuclei showed variations in size and nucleoli were distinct. Moderate amounts of necrotic material and inflammatory cells including foamy macrophages were identified in this grade of carcinomas (Figure 2). The study identified 11 carcinomas as grade 2. Grade 3 carcinomas had numerous cells that were seen dissociated without any cluster formation (Figure 3). Cells were much bigger with marked cellular and nuclear pleomorphism. Nucleoli were prominent and chroma-

tin appeared coarse. Higher numbers of atypical mitotic figures, greater amounts of necrotic debris, large numbers of inflammatory cells and syncytia formation could be observed. Cytoplasm of neoplastic epithelial cells appeared vacuolated and chromatin was coarse and granular (Figure 4-6). Four carcinomas were identified as grade 3.

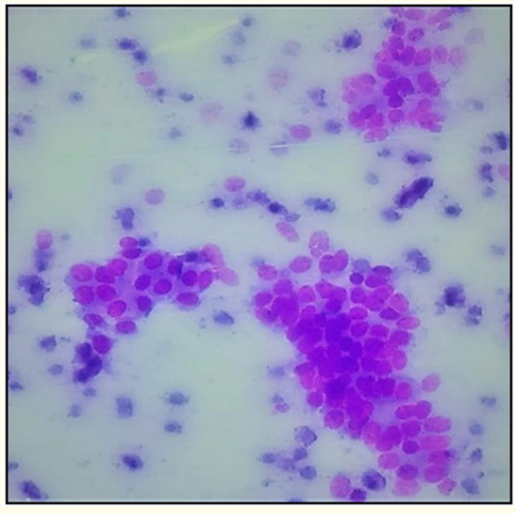

Figure 1: Grade1 tumour-clusters of uniform epithelial cells with smooth nuclear membrane. Field Stain x 400.

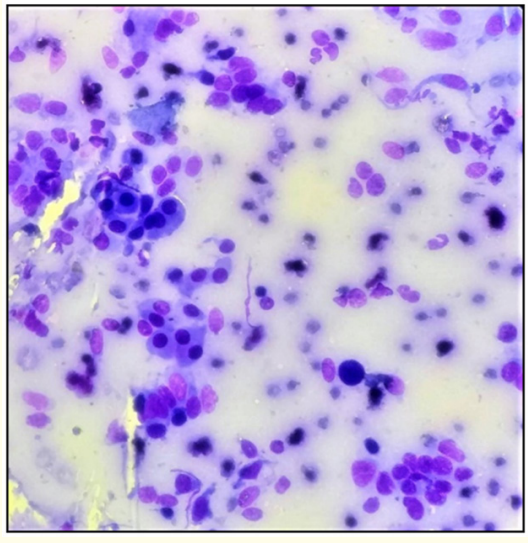

Figure 2: Grade 2 tumour- cells with moderate pleomorphism with mild cluster formation. Field Stain x 400.

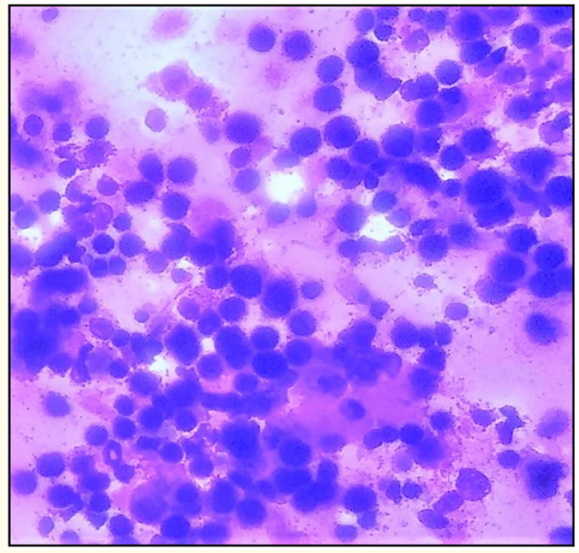

Figure 3: Grade 3 tumour - single cells with marked pleomorphism and no cluster formation. Field Stain x 400.

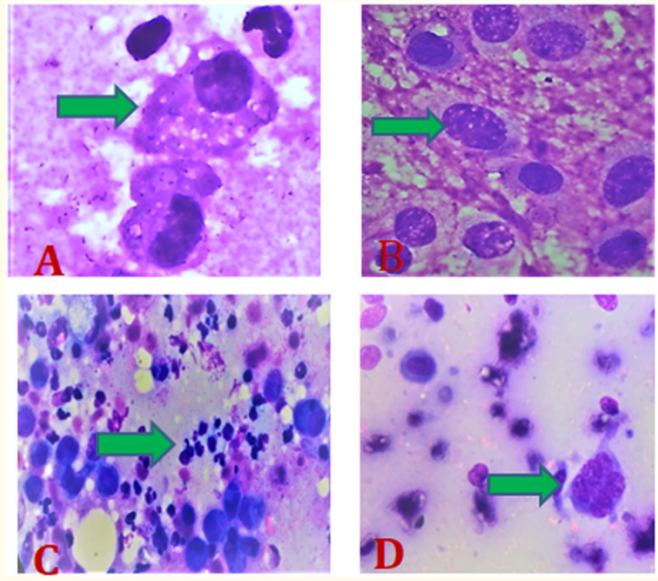

Figure 4: Cytoplasmic vacuolation B: Coarse chromatin C: Inflammatory cells D: Syncytia.

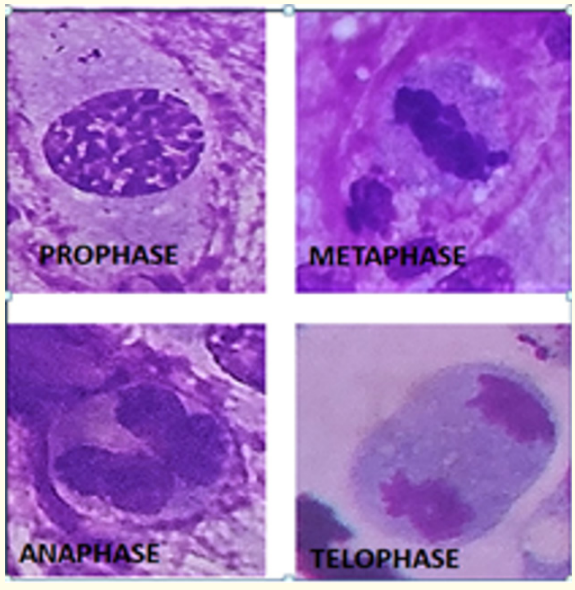

Figure 5: Cells in different stages of mitosis.

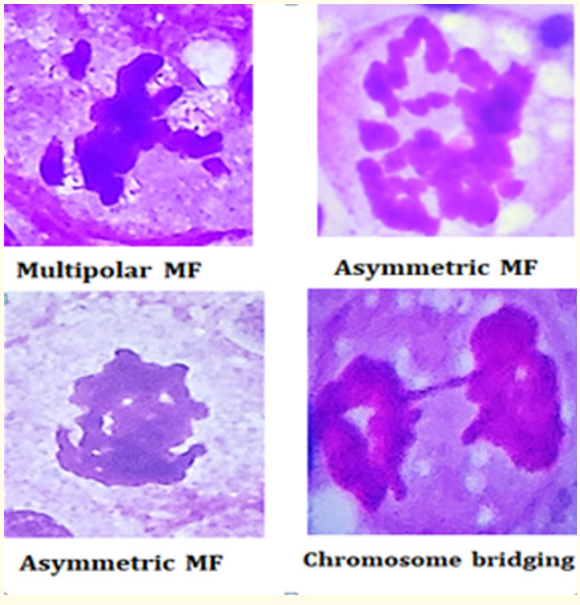

Figure 6: Atypical mitotic figures.

Histological grading identified eight numbers of grade I carcinomas with marked tubule formation, mild nuclear pleomorphism and a very low mitotic count (Figure 7). Ten carcinomas with moderate tubule formation and pleomorphism to a moderate extent were graded as II (Figure 8). Six numbers of carcinomas were of grade III with marked nuclear pleomorphism and high mitotic counts (Figure 9).

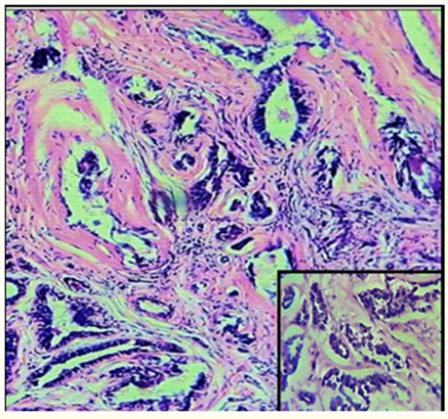

Figure 7: Grade I tumour - low nuclear pleomorphism, more tubule formation and very less mitotic count - (H and E X 100).

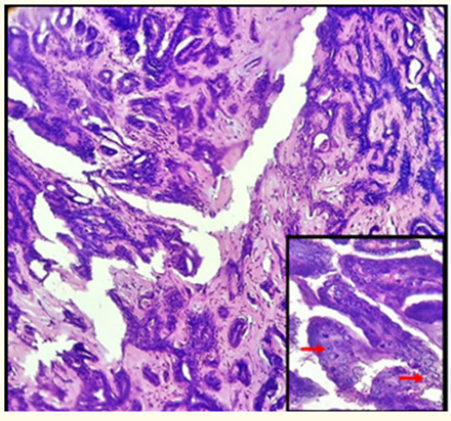

Figure 8: Grade II tumour - moderate degree of nuclear pleomorphism, reduced tubule formation and moderate mitotic count (Arrows) (H and E X 100).

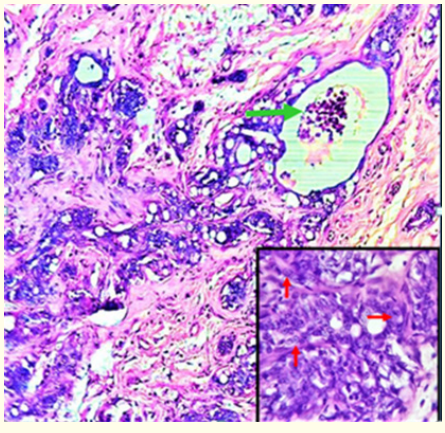

Figure 9: Grade III tumour - high nuclear pleomorphism, absence of tubule formation, very high mitotic count (red arrow) and lymphatic invasion (Green arrow). (H and E X 100).

The comparison between histological and cytological grades obtained by the analysis of 24 canine mammary carcinomas is shown in table 1.

Cytological grade	Histological grade			Total no. of cases
	I	II	III	
1	8	1		9
2		9	2	11
3			4	4
Total No. of cases	8	10	6	24

Table 1: Comparison between cytological and histological grades of CMT.

Concordance rates between two grading systems

Rates of concordance between the two grading systems were analysed individually for all the three grades and is summarised in table 2. In the present study, the concordance rate between tumours of cytological and histological Grade 1 tumours was 88.89 per cent, for Grade II tumours it was 81.82 per cent and for Grade III tumours it was 100 per cent. The absolute concordance was calculated as 87.5 per cent. Statistical analysis using Spearman Rank co-efficient revealed a significant correlation between the two systems of grading.

Grade	No. of concordant cases	Total no. of cases in cytological grading	Concordance rate (%)	Spearman rank correlation co-efficient (r)
I	8	9	88.89	r: 0.836**
II	9	11	81.82	P-value <0.001
III	4	4	100	
Total	21	24	87.5	

Table 2: Rate of concordance between cytological and histological grading systems.

** Significant at 0.01 per cent level.

Sensitivity and specificity of cytological grading system

The sensitivity and specificity of cytological grading system with respect to histological system of grading CMTs was studied and the results obtained are summarised in table 3.

It was observed that, for cytological Grade 1 tumours, the sensitivity and specificity were 100 per cent and 93.75 per cent respectively. With regard to cytological Grade 2 tumours the sensitivity was 90 per cent and specificity was 85.71 per cent, while the sensitivity was 66.67 per cent and specificity was 100 per cent in cytological Grade 3 tumours.

Cytological grade	No. of true positive cases	No. of false positive cases	No. of true negative cases	No. of false negative cases	Sensitivity	Specificity
1	8	1	15	-	100	93.75
2	9	2	12	1	90	85.71
3	4	-	18	2	66.67	100

Table 3: Sensitivity and specificity of cytological grading in relation to histological grading.

Discussion

Inspite of several similarities between CMTs and HBCs, mixed mammary tumours are rare in humans and hence, could pose a diagnostic challenge, especially when cytological analysis is carried out based on the criteria described for HBCs. Hence, smears having mesenchymal like cells suggestive of mixed mammary tumours were excluded from cytological analysis on account of the complexities and inaccuracies reported by earlier authors [5,12]. According to the observations of some previous studies, presence of mesenchymal cells was not an exclusive or pathognomonic feature of mixed mammary tumours; instead, they could be seen in other tumours like spindle cell carcinomas and complex carcinomas [1,12]. The results of these studies had shown that presence of spindle cells in cytology smears was attributable to misdiagnosis and histopathology should be taken as the mainstay for differentiation of CMTs in such cases. Considering the above aspects, only carcinomas were graded in the present study. Though CMTs mimic HBCs in various aspects, the atypia and heterogeneity of CMTs, extensive necrosis and inflammation associated with them and the challenge posed by mixed mammary tumours necessitate some

inevitable revisions in Robinson's method of cytological grading of mammary carcinomas. Hence, apart from Robinson's criteria, numerous additional features pertinent to CMTs as suggested in various other literature were also incorporated for analysis [4,8,9].

Out of the s24 mammary carcinomas considered for grading, nine were of cytological Grade 1, 11 were Grade 2 and four were Grade 3. Histological system of grading identified eight tumours as Grade I, 10 as Grade II and six as Grade III. Rates of concordance between the two grading systems were analysed individually for all the three grades. In the present study, the concordance rate between tumours of cytology Grade 1 and histology Grade I tumours was 88.89 per cent, for Grade II tumours it was 81.82 per cent and for Grade III tumours it was 100 per cent. In a study which attempted to grade the fine needle aspirates from HBC cases as described in Robinson's method, a substantial agreement between cytological and histopathological grades were obtained for Grade I and II carcinomas, while in Grade III carcinomas it was nearly 100 per cent [16]. Our study also identified 100 per cent concordance rate between cytological and histological grading systems for Grade III tumours. There are some studies on HBCs which had reported similar concordance rates by employing Robinson's criteria for cytological grading [6,7,10]. However, the cytohistological concordance rate reported in case of CMTs on using Roinson's method was much lower than that reported for HBCs, which could be largely due to the inconclusiveness arising in interpreting the mesenchymal like cells in cytological smears. In many of these reports, the concordance between cytohistological grading ranged between 27 to 45 per cent [5,12]. It was also evident from some reports that exclusion of such inconclusive cases could give a higher concordance rate even up to 94 per cent [2,4,15]. The relatively higher rate of concordance obtained in the present study could be due to the exclusion of mixed tumours as well as the inclusion of additional cytological criteria like formation of syncytia, presence of background substances and inflammatory cells in grading of carcinomas. The heterogeneity and varying degrees of atypia occurring within the same tumour could be suggested as a possible reason for discordance between cytological and histological grading in 12.5 per cent of our cases. The confusions in determining features such as nuclear margins, chromatin clumping, and granularity on cytology smears might also have contributed to the observed discordance [11].

The sensitivity and specificity of cytological grading system with respect to histological system of grading CMTs was studied and it was observed that sensitivity was 100 per cent in cytological Grade 1 tumours, while specificity was 100 per cent for cytological Grade 3 tumours. Sensitivity was the least for Grade 3 tumours while Grade 2 tumours had the lowest specificity. The possible reason for the least sensitivity of Grade 3 tumours is that cytological grading criteria for Grade 2 and 3 CMTs often overlap and in some of the studied cases, Grade 2 tumours got misdiagnosed as Grade 3 accounting for a larger proportion of false negative cases. Previously also, a similar finding of lowest sensitivity for cytological Grade 3 tumours (37.5 per cent) as compared to sensitivity of 96.96 per cent for cytological Grade 1 and 87.18 per cent for cytological Grade 2 tumours had been reported [16]. Low specificity for Grade 2 tumours could be due the relatively higher false positive cases which might have resulted from the error due to the limited area approached for sampling and the increased heterogeneity within the same tumour mass [11].

Conclusion

Cytoloical grading for designing pre-surgical therapeutic interventions is being routinely followed in HBCs, while in canine oncology the technique is less explored. Cytological evaluation of fine needle aspirates from CMT suspected cases could reliably differentiate between benign and malignant lesions, thereby aiding in pre-surgical diagnosis and formulation of adjunct therapies. Apart from that, the present study has demonstrated that cytological grading can be as effective as histological malignancy grading for canine mammary carcinomas, when employed with sufficient expertise by incorporating several additional cytological aspects over and above the basic six Richardson's criteria. Nevertheless, the increased occurrence of mixed mammary tumours in canines and the inconclusiveness that could arise in interpreting the cytological smears in such cases remains as a real challenge in cytological diagnosis. Hence, histological diagnosis and malignancy grading continues to hold an upper hand in the conclusive diagnosis and grading of CMTs.

Author Contributions

The first author carried out the work and prepared the draft. The second author supervised the execution of the work and contributed to preparation of manuscript and illustrations. The third and fourth authors helped in critical reading of cytology and histopatholoy slides and fifth author helped in the preparation and correction of the manuscript. The sixth author supervised the collection of FNAC and excision samples from canine patients.

Acknowledgement

The authors are thankful to the Dean of the college and Director of Academics and Research of Kerala Veterinary and Animal Sciences University for funding the research project.

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