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Original Contribution

QUANTITATIVE CYTOMORPHOLOGY AS AN ADDITIONAL TOOL FOR DIFFERENTIATION BETWEEN CANINE CUTANEOUS SEBACEOUS ADENOMAS AND SEBACEOUS CARCINOMAS: A PRELIMINARY REPORT

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ABSTRACT

Eight canine cutaneous sebaceous adenomas and eight canine cutaneous sebaceous carcinomas were analyzed by computer-assisted nuclear morphometry in Hemacolor stained cytological specimens. In each case, the nuclei of at least 100 neoplastic cells were measured, and the mean nuclear area (MNA), mean nuclear perimeter (MNP), minimum nuclear diameter (D min), maximum nuclear diameter (D max) and mean nuclear diameter (D mean) were calculated. The results indicated an increase of the mean values of investigated parameters from canine cutaneous sebaceous adenomas (MNA: 56.12 ± 3.55; MNP: 26.70 ± 1.06; D min: 7.62 ± 0.46; D max: 9.33 ± 0.47; D mean: 8.27 ± 0.26) to canine cutaneous sebaceous carcinomas (MNA: 80.91 ± 7.38; MNP: 32.04 ± 1.69; D min: 9.00 ± 0.35; D max: 11.16 ± 1.00; D mean: 9.97 ± 0.44). The statistical analysis revealed statistically significant differences between benign and malignant neoplastic cells ($P < 0.01$). The results indicated that the computerized morphometry could be used as an effective auxiliary tool for differential diagnosis between canine cutaneous sebaceous adenomas and carcinomas on cytologic smears.

Key words: cytology, computer-assisted morphometry, canine cutaneous sebaceous adenomas and carcinomas

INTRODUCTION

Tumours of the sebaceous glands are reported in all domestic animals, more frequently in old cats and dogs (1, 2). They are classified as hyperplasia, epithelioma, glandular adenoma, and carcinoma according to their histological features and growth (3, 4). Among tumours of the sebaceous glands, adenomas are frequently observed in dogs, whereas carcinomas are rare both in cats and dogs (4).

Quantitative cyto and histopathology have offered a numerical scale for a range of features obtained from tumour tissues, including number of area fraction of analysis objects, linear distance between objects as well as various form factors (e.g. size and shape) characterizing the objects (5). Whereas the conventional assessments in tumour pathology have focused on the readily visible features (such as the neoplastic nuclei and the chromatin texture within the nuclei, or cells density and mitotic figures), a variety of techniques have been introduced as tools for improved diagnostic accuracy and prognostic settlement. The best diagnostic measurement technique of quantitative pathology has been suggested to take advantage of the control in manual analysis and the potential provided by computer-assisted image analysis (6).

The potential diagnostic role of morphometry in discriminating benign from malignant tumours in veterinary medicine has been investigated mainly with regard to its application in histologic rather than in cytologic smears (7).

The aim of this study was to define whether the morphometric parameters MNA, MNP, D min, D max and D mean could be used as objective diagnostic criteria for differentiation between canine cutaneous apocrine adenomas

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and apocrine carcinomas on cytologic specimens.

MATERIAL AND METHODS

Tumours

The study was performed on samples from 8 cutaneous sebaceous adenomas and 8 sebaceous apocrine carcinomas obtained from 16 dogs of different breeds and age. The tumours were collected at the time of the surgical removal from dogs, presented to the Department of Surgery, Faculty of Veterinary Medicine, Trakia University, Bulgaria.

Of the 8 dogs with cutaneous sebaceous adenomas in this study, 4 were male (50 %) and 4 (50 %) were female. Their age at the time of surgical excision ranged from 8 to 13 (mean 10) years. The following breeds were represented: Cocker Spaniel (2), Poodle (2), mixed (1), Siberian Husky (1), Samoyed (1) and Dachshund (1). Of the 8 dogs with cutaneous sebaceous carcinomas 5 were male (62.5 %) and 3 (37.5 %) were female. Their age at the time of surgical excision ranged from 9 to 13 (mean 12) years and the distributions of the breeds were as followed: Cocker Spaniel (4), Terrier (2 dogs) and mixed (2).

Cytologic and histopathologic processing

Tumour cells were preoperatively obtained by fine-needle aspiration biopsy, fixed immediately with Merckofix spray® (Merck, Darmstadt, Germany) and stained with Hemacolor® (Merck, Darmstadt, Germany). The fine-needle aspiration biopsy was performed under local anaesthesia. After surgical removal all tumour's diagnoses were histopathologically confirmed according to WHO International Histological Classification of Tumours of Domestic Animals (8). The criteria for histopathological classification of investigated tumours included cellular and nuclear pleomorphism, number of nucleoli, frequency of mitosis, discreteness of cellular borders, presence of necrosis and stromal tissue.

Nuclear cytomorphometric analysis

Samples for nuclear cytomorphometry were selected on the basis of the high quality of the slides (quantity and morphology of cells, quality of stain, absence of artefacts, specimen thickness, etc.). The cytological material was analyzed with a Motic Professional B3 digital microscope coupled to a computer equipped with the Image Pro Plus® analysis system (Media Cybernetics, version 4.5.0.29 for Windows 98/NT/2000). The measurements were calibrated with the aid of a micrometer ruler (Motic). Briefly, 10 fields for each case containing neoplastic cells were selected according to quantity and morphology of the cells with x 40 objective lens. The images created by the computer system were stored in the system's digital memory, formatted as .jpeg files and displayed on the monitor screen. For each tumour 100 nuclei of cells from selected areas were measured by outlining their profiles with a computer mouse. Overlapping and fragmented nuclei, nuclear caps and nuclei showing unclear boundaries were rejected.

The nuclear parameters evaluated in this study were mean nuclear area (MNA; μm^2), mean nuclear perimeter (MNP; μm), minimum nuclear diameter (D min; μm), maximum nuclear diameter (D max) and mean nuclear diameter (D mean).

Statistical analysis

The data from computerized cytomorphometry were analyzed by one-way analysis of variance (ANOVA/LSD test) at a level of significance $P < 0.01$ (Statistica 6.0, StatSoft, Tulsa, OK, USA).

RESULTS AND DISCUSSION

The data for the investigated parameters MNA, MNP, D min, D max and D mean for each of the 16 tumours examinated are presented in **Table 1.** The mean values for the groups are given in **Table 2.** The results indicated an increase of the mean values of investigated parameters from canine cutaneous sebaceous adenomas (MNA: 56.12 ± 3.55; MNP: 26.70 ± 1.06; D min: 7.62 ± 0.46; D max: 9.33 ± 0.47; D mean: 8.27 ± 0.26) to canine cutaneous sebaceous carcinomas (MNA: 80.91 ± 7.38; MNP: 32.04 ± 1.69; D min: 9.00 ± 0.35; D max: 11.16 ± 1.00; D mean: 9.97 ± 0.44). The statistical analysis revealed statistically significant differences between benign and malignant neoplastic cells ($P < 0.01$).

In this study, we found that MNA, MNP, D min, D max and D mean differed significantly among canine cutaneous sebaceous adenomas and sebaceous carcinomas ($P < 0.01$). The mean MNA, MNP, D min, D max and D mean values in malignant tumours were higher than in benign tumours.

Table 1. *Values of the morphometric nuclear parameters in each of the examinated tumours.*

Tumours	MNA (um^2)	MNP (um)	D min (um)	D max (um)	D mean (um)
Sebaceous adenomas					
1	53.20	25.63	7.50	9.17	8.06
2	51.62	25.31	7.56	8.59	7.92
3	54.32	26.00	7.61	8.87	8.14
4	55.35	26.67	7.66	9.49	8.24
5	56.16	27.11	6.67	9.63	8.29
6	55.58	26.71	7.86	9.89	8.21
7	61.87	28.51	7.79	9.87	8.66
8	60.87	27.65	8.32	9.13	8.67
Sebaceous carcinomas					
1	77.42	31.06	8.89	10.45	9.74
2	75.26	30.69	8.98	10.17	9.63
3	79.25	31.52	8.84	11.28	9.85
4	73.42	30.54	8.74	10.54	9.55
5	74.05	30.61	8.61	10.34	9.53
6	88.19	33.52	9.76	11.19	10.42
7	86.75	33.47	9.02	12.54	10.32
8	92.93	34.95	9.17	12.77	10.69

MNA, mean nuclear area; MNP, mean nuclear perimeter; D min, minimum nuclear diameter; D max, maximim nuclear diameter; D mean, mean nuclear diameter.

Table 2. *Mean values of the morphometric nuclear parameters in the examinated canine sebaceous adenomas and carcinoma*s

Parameter	Sebaceous adenomas (n=8)	Sebaceous carcinomas (n=8)	P (<0.01)*
MNA			
Range	51.62 - 61.87	73.42 - 92.93	yes
Mean ± SD (um^2)	56.12 ± 3.55	80.91 ± 7.38	yes
MNP			
Range	25.31 - 28.51	30.54 - 34.95	yes
Mean ± SD (um)	26.70 ± 1.06	32.04 ± 1.69	yes
D min			
Range	6.67 - 8.32	8.61 - 9.76	yes
Mean ± SD (um)	7.62 ± 0.46	9.00 ± 0.35	yes
D max			
Range	8.59 - 9.89	10.17 - 12.77	yes
Mean ± SD	9.33 ± 0.47	11.16 ± 1.00	yes
D mean			
Range	7.92 - 8.67	9.53 - 10.69	yes
Mean ± SD	8.27 ± 0.26	9.97 ± 0.44	yes

MNA, mean nuclear area; MNP, mean nuclear perimeter; D min, minimum nuclear diameter; D max, maximim nuclear diameter; D mean, mean nuclear diameter.
*Comparisons were made by one-way analysis of variance (ANOVA/LSD test).

The usefulness of computer-assisted morphometry in veterinary medicine has been recognized in the diagnosis of various spontaneous canine tumours (9-19). Moreover, several studies have outlined nuclear morphometric analysis as a valuable diagnostic predictor in various cancer in small animals (10, 12, 17-19). Computer-assisted morphometry could be applied both in cytology and histology, but the cytological application is more convenient for practical purposes (7). The measurement procedure is more easily performed on cytologic than on histologic specimens, because of the more homogeneous background of cytologic smears. This leads to quick evaluation and interpretation of obtained results, which makes the method useful for practical purposes. Moreover, on cytologic smears the cells are arranged in one plane, thus their morphometric evaluation is easier (20, 21).

In conclusion, the results from our analysis indicated that the computerized morphometry could be used as an effective auxiliary tool for differential diagnosis between canine cutaneous sebaceous adenomas and carcinomas on cytologic smears. Apparently, further morphometric studies with large series are needed to evaluate in depth the quantitative features in canine cutaneous sebaceous adenomas and carcinomas.

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