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Nuclear morphometry in relation to tumor grade in canine spontaneous cutaneous squamous cell carcinomas

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Abstract: In a retrospective study on cytological specimens from 17 dogs with histologically confirmed cutaneous squamous cell carcinoma, the morphometric variables were studied and compared to tumors' nuclear grade. The morphometric parameters evaluated in this study were mean nuclear area (MNA, μm2), mean nuclear perimeter (MNP, μm), mean nuclear diameter (D mean, μm), minimum nuclear diameter (D min, μm), and maximum nuclear diameter (D max, μm). Associations between MNA, MNP, D mean, D min, and D max and tumors' grade were assessed using the ANOVA/LSD test (Statistica 6.0, StatSoft, USA) at a level of significance of $P < 0.05$. The result indicated an increase in the mean values of the nuclear parameters with increasing tumor grade. In the cases examined, significant differences in MNA, MNP, and D mean occurred between tumors in all grades. Besides, no significant differences in D max (between grade I-II and II-III) and D min (grade II-III) were found. In conclusion, nuclear morphometry could be used as an additional tool for differentiating nuclear grade in canine cutaneous squamous cell carcinomas.

Key words: Image analysis, nuclear morphometry, tumor grade, canine cutaneous squamous cell carcinomas

Squamous cell carcinoma is one of the more common malignant cutaneous tumors in the dog and the most common in the cat (1-3). It usually affects older animals and there is no known breed predisposition in either species. The most common cutaneous locations of neoplasms in the dog are the nail bed, scrotum, nasal planum, legs, and anus (4,5). Squamous cell carcinoma may be productive, forming a friable, papillary growth, or it may be erosive, forming an ulcerated lesion. The tumor is locally invasive and infiltrates the underlying dermal and subcutaneous tissue. Metastasis tends to be via the lymphatic route but the incidence of metastasis is variable. Neoplasms of the skin are usually well differentiated and slow to metastasize; at other sites, for example the nail bed of the digit, behavior can be much more aggressive (3).

Distortion of nuclear shape and nuclear enlargement has been recognized as hallmarks of cancer and component of anaplasia. Interactive computerized morphometry is a quantitative technique that measures the dimensions of nuclear size and the shape of tumor cells. It is a relatively new discipline that enables quantification of cellular

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changes taking place in various tissues. The resulting data are objective, and the technique is quickly performed using conventional microscopic analysis. The last investigations in this area showed that nuclear profiles have been suggested as useful predictors of objective malignancy grading and prognosis in various cancers in small animals (6-15).

The aim of the present study was to investigate whether quantitative measurements of nuclear variables could be used as objective diagnostic criteria for canine spontaneous squamous cell carcinomas.

Seventeen squamous cell carcinomas obtained from 17 dogs of different breeds and age were examined. The tumors were collected at the time of the surgical removal from dogs, presented to the Department of Surgery, Faculty of Veterinary Medicine, Trakia University, Bulgaria.

Samples for processing were selected on the basis of slide quality. Tumor cells were obtained preoperatively 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 by sampling cells from 4 different areas of tumor formations. After surgical removal, all tumor diagnoses were histopathologically confirmed according to WHO International Histological Classification of Tumors of Domestic Animals (16). The neoplasms were graded histopathologically according to the Broders grading system (17): 6 well differentiated (grade I), 6 moderately differentiated (grade II), and 5 poorly differentiated (grade III).

The material obtained for cytopathological processing was analyzed with a Motic Professional B3 digital microscope (Motic, China Group Co Ltd, Hong Kong, China) coupled to a computer equipped with the Image Pro Plus® analysis system (Media Cybernetics, Silver Spring, MD, USA, version 4.5.0.29 for Windows 98/NT/2000). The measurements were calibrated with the aid of a micrometer ruler (Motic®). Fields containing neoplastic cells were randomly selected in the areas of highest cellularity, with a 40× objective lens. The images created by the computer system were stored in the digital memory of the system, formatted as jpeg files and displayed on the monitor screen. Briefly, for each case, 10 microscopic fields containing neoplastic cells were randomly selected in the areas of highest cellularity, using a 40× objective lens. At least 100 neoplastic nuclei were analyzed in each case. Precautions were taken to include only intact nuclei. After selection of the proper portion of the cytological specimens and taking the digital photos, the nuclei borders were outlined using the "Draw/Merge object" function. The morphometric parameters evaluated in this study were mean nuclear area (MNA, μm^2), mean nuclear perimeter (MNP, μm), mean nuclear diameter (D mean, μm), minimum nuclear diameter (D min, μm), and maximum nuclear diameter (D max, μm).

Associations between MNA, MNP, D mean, D min, and D max and tumor grade were assessed using the ANOVA/LSD test (Statistica 6.0, StatSoft, USA) at a significance level of $P < 0.05$.

The data for the investigated parameters MNA, MNP, D mean, D max, and D min for each of the 17 tumors examined are presented in Table 1. The mean values for tumors in different grades are given in Table 2. The result indicated an increase in the mean values of the nuclear parameters with an increase of tumor grade. In the cases examined, significant differences in MNA, MNP, and D mean occurred between tumors in all grades. Besides, no significant differences in D max (between grade I-II and II-III) and D min (grade II-III) were found.

The parameters assessed by morphometry are derived from the well-known criteria used in tumor pathology: nuclear pleomorphism can be quantified by measuring of nuclear area and its deviation, whereas the shape of nuclei can be described by a set of different shape and form factors (6). The major advantage of morphometric method is the higher reproducibility and reduction of the interobserver bias, when compared to qualitative and quantitative methods usually applied in tumor diagnosis. With the development of modern image analysis and computer technologies, the morphometric assessment of nuclear parameters has been facilitated and the time expense has significantly been reduced. In consequence, morphometric analysis is a valuable method, increasing the diagnostic certainty in routine pathology (6,7).

Table 1. Mean values of the morphometric nuclear parameters in each of the examined canine cutaneous squamous cell carcinomas in different grades.

Canine cutaneous squamous cell carcinomas	MNA (mm^2)	MNP (mm)	D mean (mm)	D min (mm)	D max (mm)
	87.81	33.21	10.4	9.37	11.2
	81.87	32.5	9.98	8.3	11.45
Grade I	89.11	34.53	10.38	8.15	12.82
(n = 6)	83.99	32.59	10.16	9.14	10.95
	92.7	31.48	10.57	8.72	13.3
	89.51	34.29	10.44	9.02	12.54
	99.77	35.31	11.1	10.5	11.76
	97.78	35.69	10.92	9.15	12.89
Grade II	95.54	34.88	10.84	9.64	12.28
(n = 6)	92.78	34.36	10.68	9.56	12.17
	91.32	34.57	10.55	8.81	12.66
	104.65	36.42	11.36	10.24	12.11
	107.38	36.94	11.5	10.34	11.5
Grade III	130.82	44	13.04	8.64	13.04
(n = 5)	110.31	39.07	11.91	9.06	11.91
	110.51	37.67	11.79	10.33	11.79
	108.5	36.79	11.57	10.61	11.57

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

Table 2. Mean values of the morphometric parameters in canine cutaneous squamous cell carcinomas of different grades.

Parameter	Grade I (n = 6)	Grade II (n = 6)	Grade III (n = 5)
MNA (μm^2)	87.50 ± 3.94 (81.87-92.70)	96.97 ± 4.87 (91.32-104.65)	113.50 ± 9.76 (107.38-130.82)
MNP (μm)	33.10 ± 1.15 (31.48-34.53)	35.21 ± 0.76 (34.36-36.42)	38.89 ± 2.99 (36.79-44.00)
D mean (μm)	10.32 ± 0.21 (9.98-10.57)	10.91 ± 0.29 (10.55-11.36)	11.96 ± 0.62 (11.50-13.04)
D min (μm)	8.78 ± 0.48 (8.15-9.37)	9.65 ± 0.63 (8.81-10.50)	9.80 ± 0.88 (8.64-10.61)
D max (μm)	12.04 ± 0.96 (10.95-13.30)	12.31 ± 0.40 (11.76-12.89)	14.06 ± 1.86 (12.90-17.30)

Several studies reported a significant correlation between morphometric parameters of the nucleus and the grading of malignant tumors (6-15). Most of the investigations, however, were performed on histological specimens.

In this study, we found that MNA, MNP, and D mean are the most appropriate nuclear parameters for differentiating between different grades of canine squamous cell carcinomas. In the veterinary medical literature, there is only one report related to significance of quantitative analysis in canine squamous cell carcinomas (9). The authors investigated the diagnostic and prognostic value of nuclear histomorphometry in canine squamous cell carcinomas. The results indicated that morphometry analysis is a simple and reproducible method that could be used to provide objective diagnostic criteria for canine cutaneous squamous cell carcinomas.

In conclusion, the results of our investigations demonstrated that nuclear morphometry could be used as an auxiliary method for grading different types of canine cutaneous squamous cell carcinomas. Due to the relatively small number of the studied neoplastic growths, it is obviously necessary to perform further studies in the field of canine squamous cell carcinoma quantitative analysis. Such efforts could be, in our view, definitely rewarding, considering the benefit for our patients.

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