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Original Contribution

NUCLEAR MORPHOMETRY IN RELATION TO METASTASES 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 metastases in regional lymph nodes. 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 in non metastatic and metastatic tumours were assessed using the ANOVA/LSD test (Statistica 6.0, StatSoft, USA) at a level of significance $P < 0.05$. The correlation between nuclear morphometric parameters and metastases to the regional lymph nodes was evaluated using Pearson's correlation test ($p < 0.05$). The mean values of these parameters were significantly greater in dogs with lymph node metastases compared to parameters of tumour cells from dogs which were lymph node-negative. Significant differences in MNA, MNP and D mean were seen between metastasizing and non-metastasizing neoplastic formations. The results from our investigation showed that nuclear morphometry is an objective and reproducible procedure that could be used for evaluating the metastatic potential of canine cutaneous squamous cell carcinomas.

Key words: nuclear morphometry, canine cutaneous squamous cell carcinomas, metastases, prognosis

INTRODUCTION

Squamous cell carcinoma is one of the more common malignant cutaneous tumours in the dog and the most common in the cat (1, 2, 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 lesions. The tumour is a 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, behaviour can be much more aggressive (3).

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

MATERIAL AND METHODS

Tumours

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. Five tumours had metastases in regional lymph nodes at the time of the diagnosis.

Cytologic and histopathologic processing

Samples for processing were selected on the basis of slide quality. Tumor cells were

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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 four different areas of tumor formations. After surgical removal all tumour's diagnoses were histopathologically confirmed according to WHO International Histological Classification of Tumours of Domestic Animals (6).

Nuclear morphometric analysis

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 x 40 objective lens. The images created by the computer system were stored in the system digital memory, formatted as .jpeg files and displayed on the monitor screen **(Fig. 1)**. Briefly, for each case, 10 microscopic fields containing neoplastic cells were randomly selected in the areas of highest cellularity, using 40 x 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 with the aid of a computer mouse. 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).

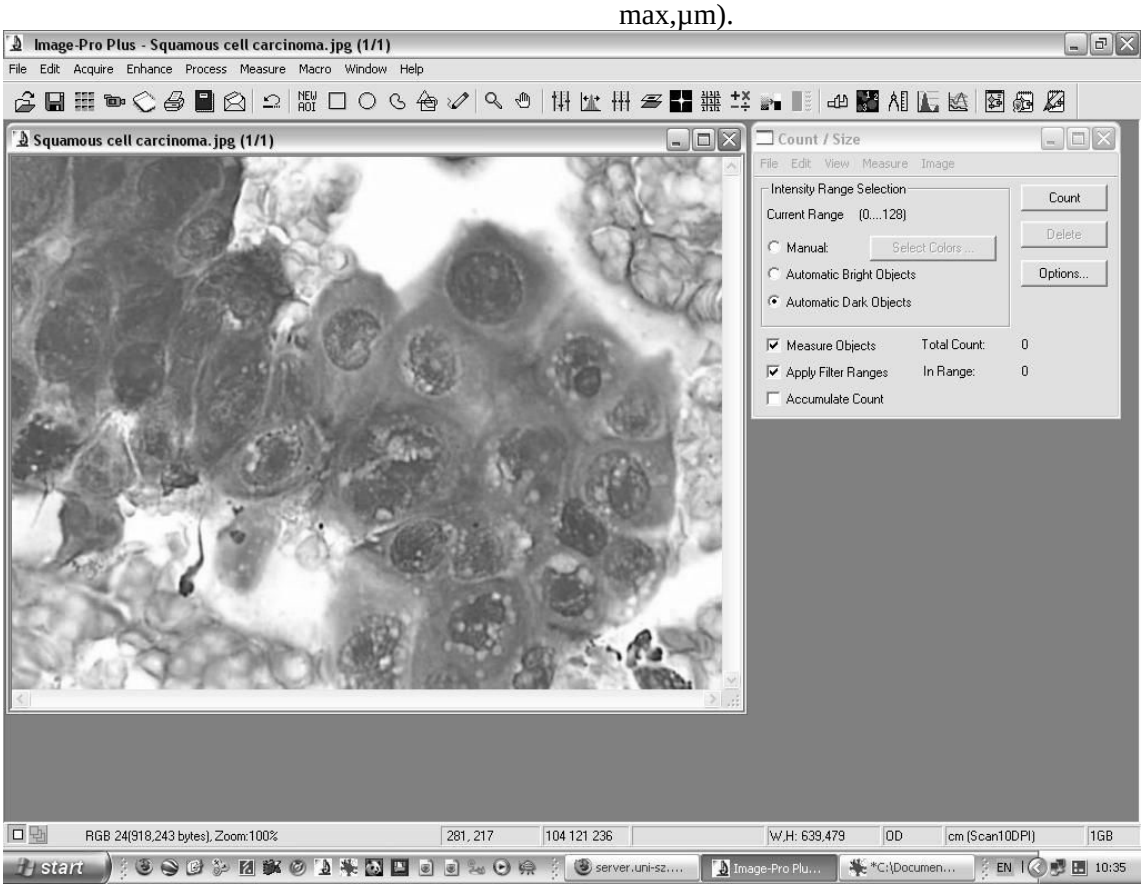

Fig. 1. Preparation for cytomorphometric evaluation.

Statistical analysis

Associations between MNA, MNP, D mean, D min and D max in non metastatic and metastatic tumours were assessed using the ANOVA/LSD test (Statistica 6.0, StatSoft, USA) at a level of significance $P < 0.05$. The correlation between nuclear morphometricparameters and metastases to the

regional lymph nodes was evaluated using Pearson's correlation test ($p < 0.05$).

RESULTS

The data for the investigated parameters MNA, MNP, D mean, D max and D min for each of the 17 tumours examined are presented in **Table 1**. The mean values of these parameters were significantly greater in dogs with lymph node metastases compared to parameters of tumour cells from dogs which were lymph node-negative **(Table 2)**. Significant differences in MNA, MNP and D mean were seen between metastasizing and non-metastasizing neoplastic formations. A significant positive correlation was also found between nuclear morphometric parameters (MNA, MNP, D mean and D max) and metastasis to regional lymph nodes **(Table 3)**.

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

Canine squamous cell carcinomas	MNA (μm^2)	MNP (μm)	D mean (μm)	D min (μm)	D max (μm)
1*	107.38	36.94	11.50	10.34	12.90
2*	130.82	44.00	13.04	8.64	17.30
3*	110.31	39.07	11.91	9.06	14.00
4*	108.51	37.67	11.79	10.33	13.13
5*	108.50	36.79	11.57	10.61	12.97
6	99.77	35.31	11.10	10.50	11.76
7	97.78	35.69	10.92	9.15	12.89
8	95.54	34.88	10.84	9.64	12.28
9	97.78	34.36	10.68	9.56	12.17
10	91.32	34.57	10.55	8.81	12.66
11	104.65	36.42	11.36	10.24	12.11
12	87.81	33.21	10.40	9.37	11.20
13	81.87	32.50	9.98	8.30	11.45
14	89.11	34.53	10.38	8.15	12.82
15	83.99	32.59	10.16	9.14	10.95
16	92.70	31.48	10.57	8.72	13.30
17	89.51	34.29	10.44	9.02	12.54

MNA, mean nuclear area; MNP, mean nuclear perimeter; D mean, mean nuclear diameter; D min, minimum nuclear diameter; D max, maximum nuclear diameter.
* Metastasizing squamous cell carcinomas.

Table 2. *Number of cases (n), mean (m) and standard deviation (Δ m) of the measured parameters for the non-metastasizing (NM) and metastasizing (M) canine squamous cell carcinomas.*

MNA (um^2)	92.24 ± 6.5 (81.87-104.65)	113.50 ± 9.76 (107.38-130.82)	p=0.00008
MNP (um)	34.15 ± 1.44 (31.48-36.42)	38.89 ± 2.99 (36.79-44.00)	p=0.0004
D mean (um)	10.61 ± 0.39 (99.98-11.36)	11.96 ± 0.62 (11.50-13.04)	p=0.00007
D min (um)	9.22 ± 0.70 (8.15-10.50)	9.80 ± 0.88 (8.64-10.61)	p=0.17
D max (um)	12.18 ± 0.72 (10.95-13.30)	14.06 ± 1.86 (12.90-17.30)	p=0.07

Table 3. *Correlation coefficients (r) between the nuclear morphometric parameters and metastases in the regional lymph nodes (p<0.05).*

	MNA	MNP	D mean (μm)	D min (μm)	D max (μm)
Metastases in the regional lymph nodes	0.81	0.76	0.83	0.35	0.62

MNA, mean nuclear area; MNP, mean nuclear perimeter; D mean, mean nuclear diameter; D min, minimal nuclear diameter; D max, maximal nuclear diameter.

DISCUSSION

The visual interpretation of the appearance of cells and tissue parts through a light microscope as a heart of medical diagnosis as carried out in cytology and histopathology (7). Human vision has a remarkable capability to rapidly interpret and recognize things in images. Computers of the other hand are very good at counting and measuring. In computerized image analysis, we are trying to give computers the capability of pattern recognition and to combine that with measurements and calculations. Image analysis today a mature science capable of creating solutions to many problems of practical interest. The lack of commonly accepted standards for how different features should be defined and studies documented in such a way that different results can be readily reproduced and compared is probably one of the main reasons why quantitative image analysis based methods have not yet fully penetrate mainline pathology practices (7).

The purpose of this study was to investigate whether quantitative measurement of nuclear parameters could provide additional information on biological behaviour of canine cutaneous squamous cell carcinomas. In the present work we found statistically significant differences in MNA, MNP and D mean between metastasizing and non-metastasizing neoplastic formations.

Increasingly abnormalities of nuclear morphometric features in parallel with tumour progression have been reported in various investigations (8). Thus, the occurrence of nuclear phenotype seems to be generalized phenomenon among neoplasia.

Several investigations in veterinary medicine have considered nuclear morphometric analysis as an useful additional predictor in canine mammary carcinomas (9, 10), feline mammary carcinomas (11), feline basal cell carcinomas (12), canine cutaneous apocrine carcinomas (13) and canine oral acanthomatous ameloblastomas (14).

In the same time, there is only one publication related to nuclear morphometry in canine cutaneous squamous cell carcinomas (15). The authors investigated the diagnostic and prognostic value of nuclear morphometry in 15 dogs with squamous cell carcinomas. The investigation was performed on histological samples. The morphometric data were examined in relation to tumour histological grade. The results indicated that nuclear morphometry analysis was a simple and reproducible method that could be used to provide objective diagnostic criteria for canine cutaneous squamous cell carcinomas.

In summary, nuclear morphometry is an objective and reproducible procedure that is relatively simply to perform. The nuclear morphometric parameters MNA. MNP and D mean were closely correlated to the malignant potential of neoplastic cells. Therefore, nuclear morphometry could provide additional information on the biological behaviour of canine cutaneous squamous cell carcinomas. Although the morphometric technique is an advance and reliable method for analyzing the nucleus, it is still not widely accepted as a routine method. Progress in the adoption of quantitative pathology is slow but it is likely that increased demands for reproducibility will lead to more widespread incorporation of morphometric methods in diagnostic decision-making

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