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Original Contribution

COMPUTER-ASSISTED MORPHOMETRY OF NUCLEAR PERIMETER IN SPONTANEOUS CANINE MAMMARY GLAND TUMOURS ON CYTOLOGIC SMEARS

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ABSTRACT

Fifty-two spontaneous canine mammary gland tumours (fibroadenomas, n = 8, tubulopapillary carcinomas, n = 9, solid carcinomas, n = 6, anaplastic carcinomas, n = 7, fibrosarcomas, n = 9, liposarcomas, n = 9 and osteosarcomas, n = 4 were analysed by computer-assisted nuclear morphometry on Hemacolor-stained cytologic specimens. One hundred nuclei per lesion were examined. Computerized cytomorphometry was performed and the values of nuclear perimeter for investigated neoplasms were assessed. The statistical analysis revealed significant differences between all tumour types (ANOVA/LSD post hoc test, $p<0.05$). The results indicated that computer-assisted nuclear morphometry could be used as an additional tool in the preoperative differentiation of benign from malignant canine mammary gland tumours.

Key words: *cytology, image analysis, computer-assisted morphometry, canine mammary gland tumours.*

INTRODUCTION

The use of computer-assisted nuclear morphometry in veterinary oncology is increasing during the past years There are several reports about the use of morphometric analysis in diagnosis of canine mast cell tumours [1, 2], feline squamous cell carcinomas [3], canine round cell tumours [4], canine and feline melanocytic tumours [5], feline mammary carcinomas [6] and canine mammary gland tumours [7, 8]. The advantages of using quantitative analysis in medicine are several. First, if applied according to strict protocols, measurement is objective and reproducible [4]. Second, it may detect some differences that are not obvious to the human eye. Third, the information obtained is well suitable for statistical evaluation. Computer-assisted morphometry can be applied both in cytology and histology, but cytological application is more convenient for practical purposes [9]. The measurement procedure is easy-performing on cytologic than on histologic specimens, because of the more homogeneous background of the cytologic smears. This leads to quick evaluation and interpretation of obtained results, which make the method useful for practical purposes. Moreover, on cytologic smears the cells are arranged in one plane, thus their morphometric evaluation is easier [10, 11, 12].

The aim of this study was to define whether the values of computer-defined nuclear perimeter could be used for differentiation of canine mammary gland tumours on cytologic smears.

MATERIALS AND METHODS

The study was performed on fifty-two spontaneous canine mammary gland tumours (fibroadenomas (n=8), tubulopapillary carcinomas (n=9), solid carcinomas (n=6), anaplastic carcinomas (n=7), fibrosarcomas (n=9), liposarcomas (n=9) and osteosarcomas (n=4). The tumours were collected at the time of surgical removal from dogs presented to the Department of Veterinary Surgery, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria. Tumour cells were obtained preoperatively by fine-needle aspiration biopsy, air dried, and stained with Hemacolor (Merck, Darmstadt, Germany). The fine-needle aspiration biopsy was performed by

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sampling cells from 4 different areas of the neoplasms. Subsequently, histopathologic diagnoses were confirmed according to Misdorp et al. [13]. A digitised image of the microscopic field was captured by a Motic Professional B3 digital microscope (*Motic, China Group Co Ltd, Hong Kong, China*). The computer was equipped with a 2.00-GHz *Celeron Intel* processor with 256 MB of RAM and a 17-inch monitor (*Samsung Electronics, Slovakia Ltd, Galanta, Slovakia*). The images created by the computer system were formatted as .jpeg files. The magnification was ×1000 and the resolution was 1024 × 768 pixels in all cases. At least 100 nuclei were analysed in each case. Precautions were taken to include only intact nuclei. Overlapping and fragmented nuclei were not measured. Computer-assisted morphometry of randomly selected nuclei was performed automatically by an image-analysis program (*Image Pro Plus 4.5.0.29 for Windows 98/NT/2000, Media Cybernetics, Silver Spring, MD, USA*). The nuclear parameter evaluated in this study was nuclear perimeter. Statistical analysis of the data was done using an ANOVA/LSD post hoc test (*Statistica 6.0, StatSoft, Tulsa, OK, USA*) at $p < 0.05$ level of significance.

RESULTS

The data about the investigated parameter are presented on **Table 1**. The comparison of nuclear perimeter between tubulopapillary carcinomas/fibrosarcomas and solid carcinomas/osteosarcomas showed less significance ($p<0.05$). Among all remaining groups the analysis revealed a high level of significant differences ($p<0.001$). The values of the mean nuclear perimeter were lowest in fibroadenomas and highest in anaplastic carcinomas. In epithelial tumours, the values of mean nuclear perimeter increased gradually as follows: fibroadenomas, tubulopapillary carcinomas, solid carcinomas and anaplastic carcinomas. In mesenchimal neoplasms, the lowest mean values of investigated nuclear parameter were found in liposarcomas, followed by fibrosarcomas and osteosarcomas.

Table 1. *Values of cytomorphometric parameter nuclear perimeter in canine mammary gland fibroadenomas, tubulopapillary carcinomas, solid carcinomas, anaplastic carcinomas, fibrosarcomas, liposarcomas and osteosarcomas. Level of significance (p) of differences among groups (ANOVA/LSD post hoc test*

Groups	***Value (μm)***		***Significance of differences (p)***						
	N	*Mean ± SD*	*FA*	*TC*	*SC*	*AC*	*FS*	*L*	*O*
Fibroadenoma (FA)	8	29.1 ± 1.97	-	***	***	***	***	***	***
Tubulopapillary carcinoma (TC)	9	34.47± 4.27	***	-	***	***	*	***	***
Solid carcinoma (SC)	6	38.62 ± 6.03	***	***	-	***	***	***	*
Anaplastic carcinoma (AC)	7	44.07 ± 5.72	***	***	***	-	***	***	***
Fibrosarcoma (FS)	9	36.08 ± 3.98	***	*	***	***	-	***	***
Liposarcoma (L)	9	32.13 ± 6.23	***	***	***	***	***	-	***
Osteosarcoma (O)	4	40.03 ± 6.46	***	***	*	***	***	***	-

**p<0.05, **p<0.01, ***p<0.001).*

DISCUSSION

The usefulness of computer-assisted histomorphometry in diagnosis of breast tumours is well known in human medicine [14, 15, 16]. Morphometric methods have also been extensively applied to the grading and prognosis of breast cancer [17, 18, 19, 20, 21], as well as for preoperative differentiation of breast neoplasms [22, 23]. In veterinary oncology there are only several reports of computer-assisted morphometric investigations in canine mammary gland tumours. All of them were performed on histologic specimens. Ciurea et al. [24] attempted to define quantitative objective criteria for diagnosis of mammary adenoma and adenocarcinoma in dogs. They examined 11 specimens from normal canine mammary tissue, 17 specimens from mammary adenoma, 18 specimens from low-grade mammary adenocarcinoma, and 15 specimens from mammary adenocarcinoma by computerized histomorphometric analysis. The investigated parameters were nuclear area, nuclear perimeter, nuclei per millimeter of basement membrane and minimal distance from cells to basement membrane. Their analysis showed that these parameters gradually increased from normal to high-grade malignancy. Destexhe et al. [25] compared two techniques for silver staining nucleolar organizer regions (AgNORs)

evaluation in 74 canine mammary tumours on histologic specimens to discriminate benign and malignant lesions. There were found significant differences between benign and malignant tumours. Investigators concluded that image analysis is a reliable, precise and convenient technique to characterize malignancy in canine mammary gland tumours. Juntes and Pogacnik [26] performed computer-assisted histomorphometric analysis to evaluated nuclear area, number of area of AgNORs per nuclear area, ratio of nuclei with five or more AgNORs, nuclear perimeter, area fraction between nuclear area and area of AgNOR. Researchers detected significant differences between normal and malignant mammary gland tissue for all measured parameters.

In our research we do not attempt to grade different types of mammary gland neoplasms or to investigate prognostic values of nuclear morphometry. Our research was connected only with searching for cytomorphometric differences between nuclear perimeters in various types of spontaneous canine mammary gland tumours. Although we found reliable differences between nuclear perimeters in all investigated tumours we think that for preoperative purposes it is enough at this stage to make morphometric characterisations whether the neoplastic lesion is benign or malignant.

In summary, our results suggest that computer-assisted morphometric analysis of nuclear perimeter could be used as an additional method for the differentiation of benign from malignant canine mammary gland tumours on cytologic smears. In future, it is important to set a threshold of nuclear perimeter value above which nucleus can be identified safely and objectively as malignant. This application would contribute to introduce automated techniques in veterinary oncology.

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