Nuclear morphometry in 36 canine spontaneous perianal gland tumours

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Keywords
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Summary
Cytological samples from 36 spontaneous canine perianal gland tumours (18 adenomas and 18 adenocarcinomas) were examined. Neoplastic cells were preoperatively obtained by fine-needle aspiration biopsy, fixed immediately with Merckofix spray® (Merck KGaA, Darmstadt, Germany) and stained with Hemacolor® (Merck KGaA, Darmstadt, Germany). Cytological smears were subjected to morphometric analysis by means of a digital microscope, PC station and image analysis software. 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), minimal nuclear diameter (D min; µm) and maximal nuclear diameter (D max). The results indicated an increase of the mean values of the nuclear parameters from canine perianal adenomas to canine perianal adenocarcinomas. The statistical analysis revealed significant differences between benign and malignant neoplastic cells ($P < 0.01$). The results in this study indicate that quantitative nuclear analysis could be used as an additional method for differentiating canine spontaneous perianal adenomas from carcinomas.

Morfometria nucleare nei tumori canini della ghiandola perianale

Parole chiave
Canino,
Analisi quantitativa,
Morfometria nucleare,
Tumori perianali.

Riassunto
Sono stati esaminati campioni citologici di 36 tumori spontanei della ghiandola perianale canina (18 adenomi e 18 adenocarcinomi). Le cellule neoplastiche sono state ottenute mediante biopsia per aspirazione con ago sottile, fissate immediatamente con Merckofix spray® (Merck KGaA, Darmstadt, Germania) e colorate con Hemacolor® (Merck KGaA, Darmstadt, Germania). Attraverso un sistema computerizzato di analisi di immagini gli strisci citologici sono stati sottoposti ad analisi morfometriche per valutare i valori medi della superficie del nuclei (MNA; μm^2) e del loro perimetro (MNP; µm) nonché la lunghezza media (D media; µm), minima (D min; µm) e massima (D max) del loro diametro. I risultati hanno indicato un aumento dei valori medi dei parametri nucleari nel passare dagli adenomi perianali canini agli adenocarcinomi perianali canini. L'analisi statistica ha rilevato differenze statisticamente significative ($P < 0,01$) tra cellule neoplastiche benigne e maligne. L'analisi nucleare quantitativa potrebbe quindi essere utilizzata come metodo aggiuntivo per la differenziazione tra adenomi e carcinomi perianali spontanei canini.

Introduction

Canine perianal gland tumours (Hepatoid gland tumours, Circumanal gland tumours) are common neoplasms that arise from modified sebaceous glands around the anus but are also present along the ventral midline from the perineum to the base of the skull, the dorsal and ventral tail, and the skin of the lumbar and sacral region (Berrocal *et al.* 1989, Goldschmidt and Hendrick 2000, Nielsen and Aftosmis 1964). These glands are often named as 'hepatoid glands', because the cells resemble hepatocytes. Circumanal adenomas

generally appear as nodular lesions affecting the perianal region (Nielsen and Aftosmis 1964). They represent the majority of canine perianal tumours (Berrocal *et al.* 1989). Siberian husky, Samoyed, Pekingese and Cocker Spaniel are most likely to develop this tumour (Goldschmidt and Hendrick 2000). The older, intact male is at high risk (Nielsen and Aftosmis 1964, Berrocal *et al.* 1989, Wilson and Hayes 1979). The tumours are slowly growing but never metastasize. Larger lesion commonly ulcerate, and hemorrhagic, keratinaceous material can often be extruded with local pressure (Berrocal *et al.* 1989, Wilson and Hayes 1979). Up to 95 % of male dogs respond completely to castration (Ross *et al.* 1991). Perianal adenocarcinomas occur much less frequently than its benign counterpart. They represent about 3-21 % of all neoplasms in this region (Brodey 1970). These tumours have metastatic potential and often spread to the regional lymph nodes. Average age of affected dogs is 11 years. Tumours occur in castrated or intact males, as well in females (Ross *et al.* 1991). They are generally not responsive to castration or to estrogen therapy (Wilson and Hayes 1979, Vail *et al.* 1990). The rate of growth of these tumours is variable. Metastases may occur via the lymphatic route to regional lymph nodes with subsequent spread to lungs and other organs (Goldschmidt and Hendrick 2000). Pathohistological evaluation is the best mean of diagnosis in canine perianal tumours. However, there is debate about how to distinguish low grade malignant tumours from circumanal adenomas because well differentiated forms can be confused with adenomas. The aim of the present study was to determine whether the quantitative nuclear analysis could be used as an additional method for differentiating canine spontaneous perianal adenomas from carcinomas.

Materials and methods

Animals

Cytological samples from 36 spontaneous canine perianal gland tumours (18 adenomas and 18 adenocarcinomas) were examined. The tumours were collected following surgical removal from dogs presented to the Department of Surgery, Faculty of Veterinary Medicine, Trakia University. Neoplastic cells were preoperatively obtained by fine-needle aspiration biopsy, fixed immediately with Merckofix spray® (Merck KGaA, Darmstadt, Germany) and stained with Hemacolor® (Merck KGaA, Darmstadt, Germany). Tumours were histopathologically confirmed according to the WHO International Histological Classification of Tumours of Domestic Animals (Goldschmidt *et al.* 1998).

Quantitative analysis

Cytological smears were subjected to morphometric analysis by means of a digital microscope[1], PC station and image analysis software[2]. The system was previously calibrated by the built-in micrometer ruler. Nuclei were randomly selected for morphometric analysis provided that they were clearly visible and intact. At least 100 nuclei were analyzed in each case. Cytologically, the hepatoid cells, characterized with abundant finely granular cytoplasm, predominated. Their nuclei resembled those of normal hepatocytes appearing round with often single or multiple, prominent nucleoli. In contrast to them, the reserve cells were significantly smaller, less numerous, and had higher nuclear/cytoplasmatic ratios and lacked features of cellular pleomorphism (Figure 1). 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), minimal nuclear diameter (D min; μm) and maximal nuclear diameter (D max). The parameters were automatically calculated by the image analysis software[2].

Statistical analysis

Data from the morphometric analysis were statistically analyzed by the Mann-Whitney U test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at a level of significance $P < 0.05$.

[1] Motic Professional B3 digital microscope (Motic, China Group Co Ltd, Hong Kong, China).

[2] Image Pro Plus® analysis system (Media Cybernetics, Silver Spring, MD, USA, version 4.5.0.29 for Windows 98/NT/2000).

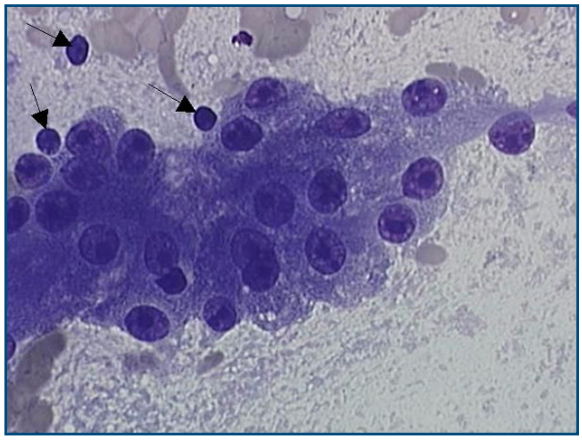

Figure 1. *Cytological picture of a hepatoid adenocarcinoma.* The reserve cells (arrows) are significantly smaller, less numerous and have a higher nuclear/cytoplasmatic ratio compared to hepatoid cells.

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Results

The data for the investigated parameters for each of the 36 tumours examinated are presented in Table I. The average numeric values of the studied parameters were significantly higher in perianal adenocarcinomas than in perianal adenomas. The results indicated an increase of the mean values of the nuclear parameters from canine perianal adenomas (MNA: 81.05 ± 3.46; MNP: 33.10 ± 2.01; D mean: 10.00 ± 0.46; D min: 8.33 ± 1.08; D max: 11.99 ± 1.67) to canine perianal adenocarcinomas (MNA: 99.15 ± 19.21; MNP: 35.88 ± 3.26; D mean: 11.04 ± 1.13; D min: 9.34 ± 1.09; D max: 12.88 ± 1.48). The statistical analysis revealed significant differences ($P < 0.01$) between benign and malignant neoplastic cells (Table II).

Discussion

The most important pathohistological criteria supporting the diagnosis of perianal adenocarcinomas is invasiveness of tumour cells into surrounding tissue. Increased nuclear pleomorphism, disorderly arrangement of cells and increased number of mitoses also are connected with malignancy (Stannard and Pulley 1978). In our previous studies (Simeonov and Simeonova 2008, Simeonova and Simeonov 2008) we investigated the prognostic value of nuclear morphometry in canine spontaneous perianal adenocarcinomas. The mean values of morphometric 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, D mean,

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

Canine hepatoid adenomas						Canine hepatoid adenocarcinomas					
Cases	MNA (μm^2)	MNP (µm)	D mean (µm)	D min (µm)	D max (µm)	Cases	MNA (μm^2)	MNP (µm)	D mean (µm)	D min (µm)	D max (µm)
1	75.01	31.12	9.58	8.47	10.68	1*	139.61	42.10	13.26	11.10	14.96
2	77.43	31.07	9.75	8.90	10.46	2*	142.43	43.15	13.57	11.08	16.41
3	75.27	30.70	9.64	8.99	10.18	3*	118.32	38.73	12.02	10.31	13.49
4	79.26	31.53	9.86	8.85	11.29	4	89.34	34.88	10.43	8.74	12.66
5	84.35	32.66	10.20	9.37	11.03	5	79.54	32.06	9.85	8.79	11.48
6	83.40	32.29	10.15	9.81	10.56	6*	122.14	39.97	12.61	10.15	14.57
7	85.99	32.77	10.32	9.84	10.67	7	85.37	33.38	10.16	8.27	12.13
8	76.30	31.55	9.69	8.58	11.08	8	88.19	33.52	10.42	9.76	11.19
9	85.24	34.12	10.13	7.91	12.69	9	87.64	35.04	10.27	7.88	13.53
10	78.92	32.75	9.91	7.68	12.40	10*	102.75	38.09	11.56	8.31	14.75
11	77.28	33.33	9.50	6.82	12.99	11*	106.49	37.00	11.49	10.81	12.74
12	82.22	32.37	10.06	9.11	11.22	12	83.53	32.71	10.12	8.94	11.41
13	85.61	32.71	10.27	9.33	10.86	13	87.86	33.33	10.39	9.33	11.22
14	80.73	35.75	9.65	6.82	14.67	14*	92.93	34.95	10.69	9.17	12.77
15	79.64	32.29	9.89	8.43	11.83	15	89.48	34.12	10.46	8.85	11.93
16	85.67	38.79	11.56	6.14	16.33	16	81.32	33.14	9.92	7.61	12.06
17	83.31	34.70	9.99	7.59	12.98	17*	94.31	34.55	10.79	10.08	11.57
18	83.26	35.23	9.82	7.25	13.90	18	93.50	35.16	10.64	8.63	12.98

MNA = mean nuclear area; MNP = mean nuclear perimeter; D mean = mean nuclear diameter; D min = minimum nuclear diameter; D max = maximum nuclear diameter.
*Metastasizing hepatoid adenocarcinomas.

Table II. *Number of cases (n), mean (m) and standard deviation (Δ m) of the measured parameters.*

Group parameter	Canine hepatoid adenomas (n=18) m ± Δm (range)	Canine hepatoid adenocarcinomas (n=18) m ± Δm (range)	Significance P
MNA (μm^2)	81.05 ± 3.76 (75.01-85.99)	99.15 ± 19.21 (79.54-142.43)	P = 0.0004
MNP (µm)	33.10 ± 2.01 (30.70-38.79)	35.88 ± 3.26 (32.06-43.15)	P = 0.004
D mean (µm)	10.00 ± 0.46 (9.50-11.56)	11.04 ± 1.13 (9.85-13.57)	P = 0.001
D min (µm)	8.33 ± 1.08 (6.14-9.84)	9.34 ± 1.09 (7.61-11.10)	P = 0.008
D max (µm)	11.99 ± 1.67 (10.18-16.33)	12.88 ± 1.48 (11.19-16.41)	P = 0.1

D max, and D min were seen between metastasizing and non-metastasizing neoplastic formations. A statistically significant correlation was found also between survival period of dogs and each of the following variables: 1) age, 2) diameter of tumour, 3) metastases in the regional lymph nodes, 4) MNA, 5) MNP, 6) D max and 7) D mean. The 87.5 % of all affected animals, that had tumour diameters > 5 cm, were already lymph node positive. A statistically significant positive correlation (p = 0.66) was found between tumour diameter and metastases to the regional lymph nodes.

In this study, we found that MNA, MNP, D mean and D min differed significantly among canine perianal adenomas and adenocarcinomas ($P < 0.01$). The mean MNA, MNP, D mean, D max and D min values in malignant tumours were higher than in benign tumours. Therefore, quantitative nuclear analysis could be helpful in differentiating these neoplasms in the dog. This is a good news along these lines. We are convinced that this method could be very useful for veterinary surgeons and their patients and we are confident that soon it will be carried out in various veterinary practices/ clinics on a routine basis.

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