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Research Article

Subtyping Canine Anal Sac Gland Adenocarcinomas by Means of Quantitative Morphology

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ABSTRACT

In this retrospective study, nuclear morphometric parameters of histopathologically confirmed anal sac gland adenocarcinomas were investigated on cytological smears in dogs and compared with the neoplastic subtype. The correlation between morphometric parameters and histopathological subtype was determined using Mann-Whitney U test (Statistica 6.0, StatSoft, USA) at level of significance $P<0.05$. There were statistically significant differences between all studied morphometric parameters of tubular, rosette and solid anal adenocarcinoma patterns. The results of this preliminary study indicated that nuclear morphometry could be used as an auxiliary method for subtyping canine anal sac gland adenocarcinomas.

Key Words: Cytology, image analysis, nuclear morphometry, histopathological subtypes, canine anal sac gland adenocarcinomas

ÖZET

ANAL KESE BEZİ ADENOKARSİNOMALARININ KANTİTATİF MORFOLOJİ YÖNTEMİ İLE SUBTİPLENDİRİLMESİ

Bu retrospektif çalışmada, köpeklerde histopatolojik olarak doğrulanmış anal kese bezi adenokarsinomalarının nükleer morfometrik parametreleri sitolojik smear yöntemiyle incelenmiş ve neoplastik subtiple karşılaştırılmıştır. Morfometrik parametreler ve histopatolojik subtip arasındaki korrelasyon, önem derecesi $P<0,05$ düzeyinde Mann-Whitney U testi (Statistica 6,0, StatSoft, USA) kullanılarak belirlenmiştir. Tubüler, rozet ve kistik anal adenokarsinoma çeşitlerinin çalışılan tüm morfometrik parametreleri arasında istatistiki açıdan önemli farklılıklar bulunmuştur. Bu ön çalışmanın sonucu olarak nükleer morfometri yönteminin köpeklerde görülen anal kese bezi adenokarsinomalarının subtiplendirilmesi amacıyla yardımcı bir yöntem olarak kullanılabileceği söylenebilir.

Anahtar Kelimeler: Sitoloji, görüntü analizi, nükleer morfometri, histopatolojik subtipler, kanin anal kese bezi adenokarsinomaları

Introduction

Anal sac gland adenocarcinomas are the commonest malignant perineal neoplasms in dogs. They account for about 2% of all skin tumours in this species (Goldschmidt and Hendrick, 2002). Animals from 5 to 15 years of age are affected, most frequently between 7-12 years (Goldschmidt and Hendrick, 2002). The tumours originate from perianal apocrine glands and usually, are unilaterally located (Goldschmidt and Zoltowski, 1981). According

to some researchers, adult female dogs are mainly affected (Goldschmidt and Zoltowski, 1981; Ross et al., 1993), while others do not report any sex-related predisposition (Bennet et al., 2002; Goldschmidt and Shofer, 1993). Canine anal adenocarcinomas are highly malignant. They affect surrounding tissues and metastasize at a very early stage, even when small in size (Bennet et al., 2002; Meuten et al., 1981; Ross et al., 1991). Metastases spread to regional lymph nodes and afterwards, in lungs, liver and spleen (Bennet et al., 2002; Meuten et al., 1981; Ross et al., 1991) but rarely in other organs (Brisson et al., 2004).

Histopathological subtypes of anal adenocarcinomas are three (Goldschmidt and Hendrick, 2002). Solid tumours are with round to oval normochromatic or hyperchromatic nuclei, convex nucleoli and few amount of eosinophilic cytoplasm. Cellular nuclei of the rosette type are located basally and contain a small amount of apically situated eosinophilic cytoplasm. Tubular formations are with large lumens, lined by cubic epithelial cells. The cytoplasm of cells is profuse, and the nuclei - hyperchromatic.

The purpose of the present study was to evaluate the potential of quantitative morphology for differentiation of canine anal sac gland adenocarcinoma subtypes.

Materials and Methods

Case material

The study was performed on 9 spontaneous canine anal sac gland adenocarcinomas obtained from 9 dogs of different breeds and age (Table 1). 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. The post-operative follow up of all animals was two years.

Table 1. Signalment, clinical and postoperative follow-up data of dogs with anal sac adenocarcinomas.
Tablo 1. Anal kese adenokarsinomları olan köpeklerin eşkali, klinik ve ameliyat sonrası takip verileri.

Tumours	Breeds	Age (years)	Sex	Tumour's type	Tumour diameter	Metastases in the regional lymph nodes	Survival in months*
Anal sac gland adenocarcinomas							
1	English Cocker Spaniel	10	F	Solid	>5 cm	Yes	6
2	Rottweiler	12	F	Solid	>5 cm	Yes	7
3	Mixed	9	F	Rosette	>5 cm	Yes	10
4	German Shepherd	8	M	Tubular	<5 cm	No	18
5	German Shepherd	10	F	Rosette	>5 cm	Yes	12
6	Mixed	7	F	Tubular	<5 cm	No	21
7	Mixed	8	M	Rosette	>5 cm	No	14
8	English Cocker Spaniel	6	M	Tubular	<5 cm	No	19
9	Mixed	14	F	Solid	>5 cm	Yes	9

*Survival period was defined as the time from first detection of the tumour to either the time of death or the date on which the dog was known to be alive.

Cytologic and histopathologic processing

Tumour cells were preoperatively obtained by FNAB, fixed immediately with Merckofix spray® (Merck, Darmstadt, Germany) and stained with Hemacolor® (Merck, Darmstadt, Germany). After surgical removal all tumour's diagnoses were histopathologically confirmed according to WHO International Histological Classification of Tumours of Domestic Animals (Goldschmidt et al., 1998). The criteria for histopathological classification of investigated tumours included cellular and nuclear pleomorphism, number of nucleoli, frequency of mitosis, discreteness of cellular borders, invasion of adjacent stroma, presence of necrosis and stromal tissue (Figure 1).

Nuclear cytomorphometric analysis

Samples for nuclear cytomorphometry were analyzed by means of trinocular digital microscope [Motic Professional B3 digital microscope (Motic, China Group Co Ltd, Hong Kong, China)] and microscopic image analysis software [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). After visualization of findings, smear areas were randomly selected for morphometric analysis on the basis of the quality of findings (Figure 2). A minimum of 100 intact cell nuclei were analysed from each patient. The following morphometric parameters were determined: mean nuclear area (MNA; µm2), mean nuclear perimeter (MNP; µm), mean nuclear diameter (MND mean; µm), minimum nuclear diameter (D min; µm), and maximum nuclear diameter (D max; µm).

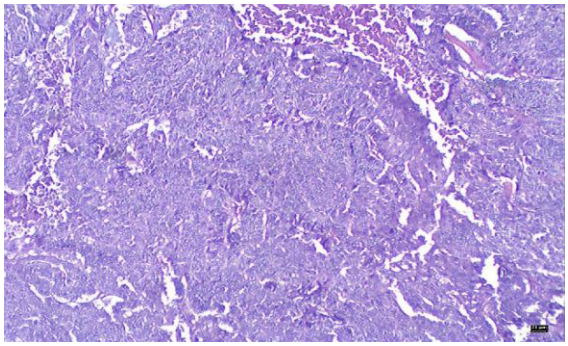

Figure 1. Histopathological view of canine anal sac gland adenocarcinoma. H/E staining.

Şekil 1. Kanin anal kese bezi adeno-karsinomalarının histopatolojik görüntüsü. H/E boyama.

Statistical analysis

The data from computerized cyto-morphometry were analyzed by statistically processed by the Mann-Whitney U test (Statistica 6.0, StatSoft, Tulsa, OK, USA) at a level of significance $P<0.05$.

Results

The dogs included in this study were Mixed-breed (4), German shepherd (2), English Cocker spaniel (2) and Rottweiler (1). Their age varied between 6 and 14 years (mean 9.33 years) (Table 1). Mean values of morphometric parameters increased from tubular (MNA, 82.45±3.03, MNP, 32.37±0.31, D mean, 10.06±0.20, D min, 9.07±0.26, D max, 11.19 ± 0.30) to rosette (MNA, 90.80±3.50, MNP, 34.41±0.81, D mean, 10.54±0.19, D min, 9.14±0.41, D max, 12.33±0.72) and solid (MNA, 111.94±10.16, MNP, 38.15±1.97, D mean, 11.85±0.71, D min, 10.21±0.07, D max, 13.35±1.22) adeno-carcinoma subtypes (Tables 2 and 3).

The statistical analysis showed significant differences in all studied parameters.

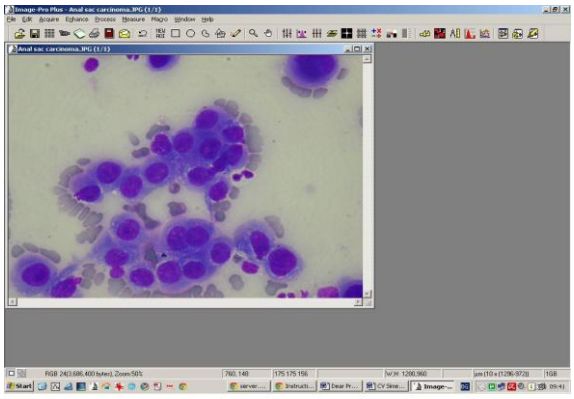

Figure 2. Cytological finding. The main menu of the software used (Image Pro Plus®).

Şekil 2. Sitolojik bulgular. Kullanılan yazılımın ana menüsü (Image Pro Plus®).

Discussion

New methods for medical diagnostics and typing of neoplasms are continuously sought worldwide. One of available options is morphometry (Baak, 1991; Baak and Tosi, 1991). It helps the identification of inter-object differences, which may remain invisible to the naked eye. Modern quantitative analysis uses computer systems for microscopic image analysis. Thus, the analytical procedures are substantially simplified allowing for the rapid introduction of the method in research laboratories (Hamilton and Allen, 1995).

Table 2. Mean values of the morphometric nuclear parameters in each of the examined subtypes of canine anal sac adenocarcinomas.

Tablo 2. Kanin anal kese bezi adenokarsinomalarının alt tiplerine ait morfometrik nükleer parametrelerin ortalama değeri.

Canine anal sac adenocarcinomas	MNA (μm^2)	MNP (μm)	D mean (μm)	D min (μm)	D max (μm)
Tubular type (n=3)	79.54	32.06	9.85	8.79	11.48
	82.21	32.36	10.05	9.10	11.21
	85.60	32.70	10.26	9.32	10.88
Rosette type (n=3)	92.93	34.95	10.69	9.17	12.77
	92.73	34.81	10.62	8.71	12.73
	86.75	33.47	10.32	9.55	11.50
Solid type (n=3)	101.80	36.05	11.20	10.18	12.13
	111.86	38.44	11.72	10.30	13.35
	122.14	39.97	12.61	10.15	14.57

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

Table 3. Mean values of the morphometric parameters in different subtypes of canine anal sac adenocarcinomas.

Table 3. Kanin anal kese adenokarsinomların farklı tiplerinde morfometrik parametrelerin ortalama değerleri.

Parameter	Tubular type (n=3)	Rosette type (n=3)	Solid type (n=3)
MNA (μm^2)	82.45 ± 3.03 (79.54-85.60)	90.80 ± 3.5 (86.75-92.93)	111.94 ±10.16 (101.80-122.14)
MNP (μm)	32.37 ± 0.31 (32.06-32.70)	34.41 ± 0.81 (33.47-34.95)	38.15 ± 1.97 (36.05-39.67)
D mean (μm)	10.06 ± 0.20 (9.85-10.26)	10.54 ± 0.19 (10.32-10.69)	11.85 ± 0.71 (11.20-12.61)
D min (μm)	9.07 ± 0.26 (8.79-9.32)	9.14 ± 0.41 (8.71-9.55)	10.21 ± 0.07 (10.15-10.30)
D max (μm)	11.19 ± 0.30 (10.88-11.48)	12.33 ± 0.72 (11.50-12.77)	13.35 ± 1.22 (12.13-14.57)

There are several reports in specialised veterinary medicine literature concerning the use of computer morphometry for evaluation of canine neoplasm's malignancy. Strefezzi et al. (2003) have studied quantitatively 24 spontaneous mastocytomas. The authors established that direct morphometric parameters (area, circumference, diameter) could be used for histopathological grading of tumours. On cytological smears however, significant differences were only detected between grade I and III tumours. There were no statistically significant differences between groups with regard to indirect nuclear parameters (roundness and factor of regularity).

Maiolino et al. (2005) performed a histomorphometric study on 35 mastocytomas with various extent of differentiation. There were statistically significant differences between grade I and III tumours ($P<0.01$), but not between grades I and II ($P>0.01$). The researchers concluded that histomorphometry could be used for grading canine mastocytomas.

Having investigated 15 dogs with skin squamous cell carcinomas, Maiolino et al.

(2002) demonstrated that computer morphometry could serve as an objective tool for diagnostics and prognosis of this cancer type. This was later confirmed in another study (Simeonov, 2009).

In veterinary medicine, only two reports have assessed the relationship between morphometry and histopathological subtyping of neoplastic growths. One of studies provides evidence that morphometry could distinguish between the different histopathological subtypes of basal carcinomas in cats (Simeonov and Simeonova, 2008). It partially confirms the results of Bierhoff et al. (2003) for the same type of neoplasms in humans. According to latter study, human basal carcinomas could be subtypes by morphometric analysis, but the differentiation between recurrent from non-recurrent growths was not possible. Another study on the subject suggested that although morphometry could be useful in distinguishing the different subtypes of feline mammary carcinomas, it could not serve as a reliable prognostic factor of the outcome (Simeonov and Simeonova, 2009).

In a previous study (Simeonov and Simeonova, 2008) we have established that the prevalence risk of canine anal adenocarcinomas increased with age. Most patients with malignancies were female (63.63%), in agreement with the studies of Goldschmidt and Zoltowski (1981) and Ross et al. (1991). Furthermore, a correlation between the survival and the gender was reported, but not between the survival and the breed of dogs with cancer growths. The survival period was substantially shorted in dogs with malignant neoplasms >5 cm in diameter (mean survival time 9.42 months) compared to those with neoplasms <5 cm (mean survival time 18.75 months). Moreover, 85.71% of affected dogs with tumour size >5 cm presented already metastases in regional lymph nodes. That is why we believe that the diameter of the tumour and the presence of metastases are among the primary clinical predictors of the outcome in canine anal adenocarcinomas.

In conclusion, the results of this study suggested that the quantitative subtyping of canine anal adenocarcinomas is possible. More detailed studies in a large patient cohort are needed to confirm or reject the obtained results.

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