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Research Article

Pathomorphological approach on canine sebaceous tumors

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ABSTRACT

Sebaceous neoplasms are among the most frequently observed skin tumors in dogs. The histological classification of sebaceous glands (including Meibomian glands) is based on the sebocyte and basaloid reserve cell components. In this study aiming to examine sebaceous tumors in detail, 28 biopsies diagnosed with sebaceous neoplasia, including Meibomian gland neoplasia, were examined and diagnosed as adenoma (n:9), epithelioma (n:12), and carcinoma (n:7). Histopathologically; cell ratios (sebaceous/basal), appearance (diffuse/multilobular), presence of ulcers, necrosis, inflammation, pleomorphism and mitosis were evaluated. Ki-67 biomarker was used immunohistochemically to assess cell division. Based on histopathological and immunohistochemical examinations, it was determined that epitheliomas would rather be classified as low-grade malignancies due to their noticeable mitotic activity and should be investigated in more detail.

INTRODUCTION

Sebaceous gland tumors originate from the sebaceous glands in dermis and are among the most frequently observed tumors of dogs. They represent the third most common type of skin tumors, accounting for 22-35% of all cutaneous epithelial tumors (Vail and Withrow, 2007). They are classified as sebaceous adenoma, sebaceous ductal adenoma, sebaceous epithelioma, and sebaceous carcinoma according to their histological appearances. In adenomas, the main tumor cells are sebocytes with fewer (>50%) basaloid reserve cells and a multilobulated appearance. In sebaceous ductal adenomas, many keratinized ducts and few sebocytes and basaloid cells are seen. Sebaceous epitheliomas are characterized by a dominant population of basaloid reserve cells with few sebocytes and ducts. This type is considered a low-grade malignancy tumor, with a high mitotic activity and minimal pleomorphism. On the other hand, carcinomas consist of sebocytes showing pleomorphism with few reserve cells and ducts and a multilobulated appearance (Hendrick, 2016; Goldschmidt and Goldschmidt, 2017).

Meibomian neoplasms arise from the tarsal glands on the inner eyelid. These glands are modified sebaceous glands and classification of their tumors is similar to sebaceous neoplasms (Goldschmidt and Goldschmidt, 2017).

Histopathological features and particularly the epithelial cell ratio (sebaceous/basaloid) are of non-negligable importance for classification of sebaceous and meibomian gland tumors. Besides, circumscription, ulcer, necrosis, inflammation, pleomorphism and mitosis are considered (Costa et al., 2020).

Ki-67 is the most commonly used marker to assess proliferation in tumors. MKI67 is a protein expressed in all nuclei during the active phases of the cell cycle. Ki-67 is used to detect MKI67. It is stated that the higher Ki-67 ratio a tumor shows, the more malignant characteristics it has. (Scholzen and Gerdes, 2000; Sun and Kaufman, 2018).

In this study, 28 cases from 25 dogs submitted to our department and diagnosed with sebaceous and meibomian gland tumors were examined, with detailed evaluation of histopathological features and cell proliferation using Ki-67.

MATERIALS and METHODS

Sections were taken from paraffin blocks prepared from 28 biopsies in total, that were brought from the animal hospital of the faculty and private clinics. Hematoxylen-Eosin (HE) and trichrome stainings were performed and epithelial cell ratio (sebaceous/basaloid), diffuse/multilobular presentation, ulcer, necrosis, presence of inflammation, pleomorphism and mitosis were evaluated.

Immunohistochemically, sections taken on adhesive (3 aminopropyltriethoxysilane [APES]) slides were treated according to the kit procedure with avidine-biotine peroxidase method. For antigen rectrieval, boiling Citrate Buffer was used in steamer for 20 minutes. Anti-ki67 (1:100) (ThermoScientific,

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SP6) was incubated at 37°C for 1 hour. AEC was used as chromogen, and Mayer's Hematoxylin was used for couterstaining. Nuclear positive stainings were evaluated under the light microscope.

Nuclear pleomorphism (graded as 0,1,2,3 respectively), mitotic index (graded as -, +,++,+++ respectively) and Ki-67 (graded as +,++,+++ respectively) were assessed semi-quantitatively under x400 magnification.

RESULTS

In the study, sebaceous/meibomian tumors were most commonly observed in the following breeds: Cocker spaniel (n:6), Golden Retriever (n:5), Labrador retriever (n:2), Terrier (n:2), mixed breeds (n:2), Alaska malamute (n:1), German Shepherd (n:1), and Siberian Husky (n:1). Breed information was not available for five dogs. Sebaceous/meibomian tumors were observed in dogs aged between 2 and 16 years, with an average age of 9.6 years. Twenty-eight cases were observed in 25 animals (tumors occurred in two different locations in three animals). Of these, 15 were male, 8 were female, and gender information was not provided for two. Twenty-one of 28 tumors were located on the head and neck with 14 occuring on and around the eye and 10 of them being meibomian tumors. Of the remaining 7 head-neck located tumors, 4 were on the ear region. Information about the animals, biopsies and diagnoses are given in Table 1.

Biopsies taken from the eye and the skin were evaluated together with the prefix meibomian- used for tumors originating from the eye glands, and sebaceous- used for the tumors originating from the fat glands (including periorbital region).

Table 1. Breed, gender, age, localization and diagnostic data of the cases.

Case number	Dog data	Mass data	Diagnose
1.	Alaskan malamute, Male, 8 years old	Under left eye	Sebaceous carcinoma
2.	*No information*, Male, 14 years old	Right cheek Right ear canal entrance	Sebaceous epithelioma Sebaceous epithelioma
3.	*No information*, Female, 13 years old	Behind right ear	Sebaceous epithelioma
4.	*No information*, Male, No information	No information	Sebaceous gland adenoma
5.	*No information*, Female, 10 years old	Eye	Meibomian epithelioma
6.	Golden retriever, Male, 10 years old	Left eyelid	Meibomian adenoma
7.	*No information*, Male, 7 years old	Right eyelid	Sebaceous carcinoma
8.	Cocker Spaniel, Female, 12 years old	Foot	Sebaceous gland adenoma
9.	Terrier, Female, 16 years old	Forehead	Sebaceous epithelioma
10.	Labrador retriever, Male, 10 years old	Lower eyelid	Meibomian epithelioma
11.	Golden retriever, Male, 10 years old	Left upper eyelid	Meibomian adenoma
12.	Golden retriever, D, 10 years old	Upper eyelid	Sebaceous carcinoma
13.	German shepherd, Male, 2 years old	Eyelid	Sebaceous carcinoma
14.	Cocker Spaniel, Female, 12 years old	Corner of eye Ear	Meibomian epithelioma Sebaceous epithelioma
15.	Melez, Male, 6 years old	Skin	Sebaceous gland adenoma
16.	Cocker Spaniel, Male, 10 years old	Ear	Sebaceous epithelioma
17.	Golden retriever, Male, 10 years old	Eye	Sebaceous gland adenoma
18.	Melez, *No information*, 3 years old	Nape	Sebaceous carcinoma
19.	Siberian Husky, Male, 10 years old	Right eyelid	Meibomian carcinoma
20.	Cocker Spaniel, Male, 9 years old	Left eyelid	Meibomian carcinoma
21.	Terrier, Female, 10 years old	Fossa paralumbalis	Sebaceous gland adenoma
22.	Cocker Spaniel, Female, 11 years old	Skin	Sebaceous gland adenoma
23.	Cocker Spaniel, Male, 8 years old	Skin Eye	Sebaceous gland adenoma- Meibomian epithelioma
24.	Labrador retriever, Male, 9 years old	Eyelid	Meibomian epiteliyoma
25.	Golden retriever, Female, 11 years old	Eye corner	Meibomian epithelioma

Among the 28 biopsies examined, 9 were adenomas, 12 were epitheliomas, and 7 were carcinomas. In aminals with tumors in two different regions, two dogs had the same diagnosis for both tumors, while one had epithelioma on the eye and ademona on the body.

In histopathological evaluation; based on the sebaceous/basal epithelium ratio, 5 of the sebaceous/meibomian adenomas (55.5%) were graded as 3/1, while 4 (44,4%) were graded as 3/0 (Figure 1). All of the epitheliomas except for one (n:11, 91,6%) had an epithelial ratio of 1/3 (Figure 2 and Figure 3). The ratio was 1/2 in one case (8.3%). Among carcinomas, all but one had a ratio of 3/1 (n: 6, 85.7%), (Figure 4) while one case (%14.2) had the ratio of 2/2.

When pleomorphism was considered,6 of the adenomas (n=9) 6 (66.6%) had the score of 0, and 3 (50%) had the score of 1. The score was 0 in only one of the epithelioma cases (n=12) (8.33%), while the score was 1 in 8 (66.6%) cases (Figure 2), and 2 in 3 (25%) cases (Figure 3). Six of the carcinomas (n=7) (85.7%) scored 2, while one case scored 3 (14.2%).

Cells had a multilobular pattern in all the adenomas except for one (n=8), which had a diffuse pattern. Nine of the epitheliomas presented a multilobular pattern (Figure 2 and Figure 3) while 3 had a diffuse pattern (Figure 4). Neoplastic cells presented a diffuse pattern in all cases with carcinoma (n=7).

In terms of cyst formation, 4 of the adenomas, 11 of the epitheliomas and 4 of the carcinomas had cysts. Ulceration was reported in 3 adenomas, 6 epitheliomas, and 4 carcinomas. Only one adenoma, 7 epitheliomas and 5 carcinomas had necrosis. All cases with necrosis were accompanied by ulceration and inflammation. Regardless of classification, most cases (22/28) showed inflammatory changes. Among these inflammations, neutrophils were predominant in the areas of ulceration and necrosis, while interstitial sites in tumoral region mostly contained mononuclear cells. Hemorrhage was seen in 12 cases (adenoma:1, epithelioma:7, carcinoma:4). Histopathological evaluations are given in Table 2.

Histopathologically, mitotic activity was severe (+++) (Figure 2 and Figure 3) in epithelioma, intermediate (++) in carcinoma, and rarely seen in adenomas.

Immunohistochemically, Ki-67 was moderatly positive (++) in epithelioma and carcinoma, while it was mild (+) in adenomas. (Figure 5).

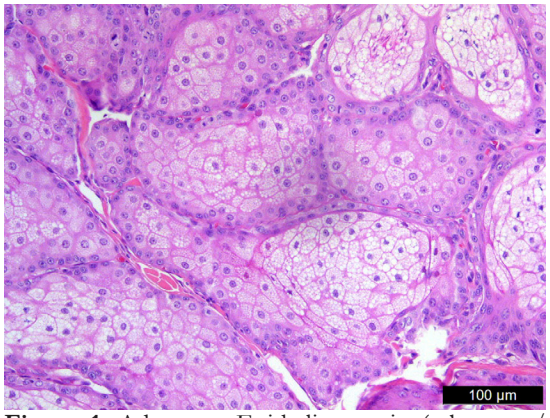

Figure 1. Adenoma. Epithelium ratio (sebaceous/basaloid): 3/0 multilobular appearance, HE.

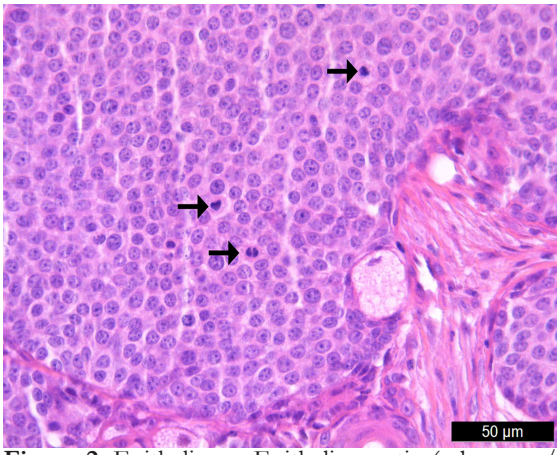

Figure 2. Epithelioma. Epithelium ratio (sebaceous/basaloid): 1/3, Nuclear pleomorphism:1, multilobular appearance, mitotic figures (+++) (arrows), HE.

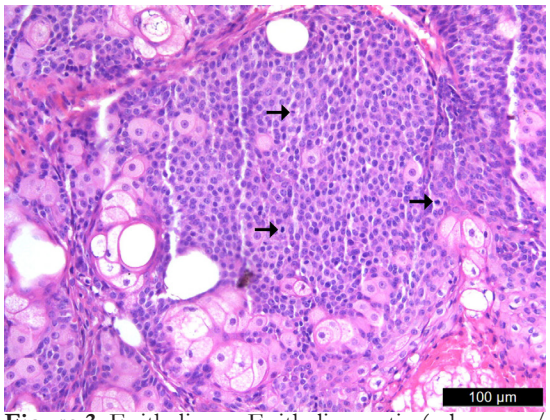

Figure 3. Epithelioma. Epithelium ratio (sebaceous/basaloid): 1/3, Nuclear pleomorphism:2, multilobular appearance, mitotic figures (+++) (arrows), HE.

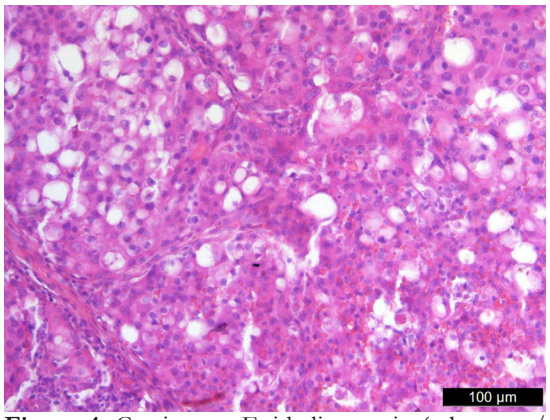

Figure 4. Carcinoma. Epithelium ratio (sebaceous/basaloid): 3/1, diffuse appearance, HE.

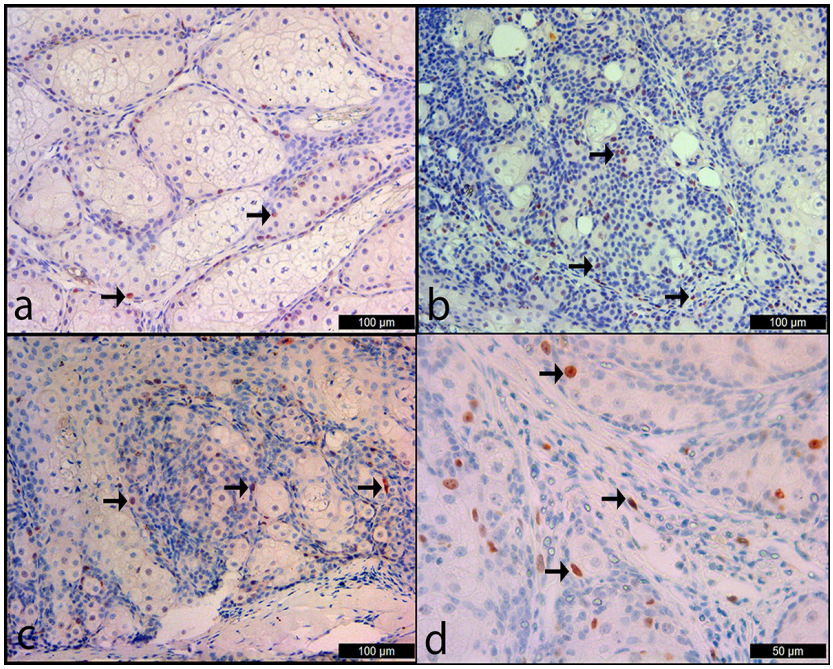

Figure 5. Immunohistochemical grading of sebaceous gland tumors for Ki-67 marker. Nuclear positivities (arrows), a) Adenoma (+), b) epithelioma (++) and c,d) carcinoma (++).

Table 2. Evaluation of criteria used in histopathological examination of the sebaceous tumors according to the cases.

No	Epithelium ratio (sebaceous/basal)	Nuclear pleomorphism (0, 1, 2, 3)	Diffuse (D) / Multilobular (ML)	Cyst(Yes/No)	Ulceration (Yes/No)	Necrosis (Yes/No)	İnflammation (Yes/No)	Hemorrhage (Yes/No)	Diagnose
1.	3/1	2	D	+	+	+	+	+	Sebaceous carcinoma
2.	1/3	0	ML	+	+	+	+	+	Sebaceous epithelioma
	1/3	2	ML	+	+	+	+	+	Sebaceous epithelioma
3.	1/3	2	ML	+	+	+	+	+	Sebaceous epithelioma
4.	3/0	0	ML	+	-	-	+	-	Sebaceous gland adenoma
5.	1/2	1	ML	-	-	-	+	-	Meibomian epithelioma
6.	3/0	1	ML	-	-	-	+	+	Meibomian adenoma
7.	2/2	2	D	+	-	-	-	-	Sebaceous carcinoma
8.	3/0	0	ML	+	+	+	+	-	Sebaceous gland adenoma
9.	1/3	2	ML	+	+	+	+	+	Sebaceous epithelioma
10.	1/3	1	ML	+	-	-	+	-	Meibomian epithelioma
11.	3/1	1	D	+	-	-	+	-	Meibomian adenoma
12.	3/1	3	D	+	+	+	+	+	Sebaceous carcinoma
13.	3/1	2	D	-	+	+	+	-	Sebaceous carcinoma
14.	1/3	1	ML	+	+	+	+	-	Meibomian epithelioma
	1/3	1	ML	+	+	+	+	-	Sebaceous epithelioma
15.	3/1	0	ML	-	-	-	-	-	Sebaceous gland adenoma
16.	1/3	1	ML	+	+	+	+	+	Sebaceous epithelioma
17.	3/1	1	ML	-	-	-	-	-	Sebaceous gland adenoma
18.	3/1	2	D	-	+	+	+	+	Sebaceous carcinoma
19.	3/1	2	D	-	+	+	+	+	Meibomian carcinoma
20.	3/1	2	D	+	-	-	+	-	Meibomian carcinoma
21.	3/0	0	ML	-	+	-	+	-	Sebaceous gland adenoma
22.	3/1	0	ML	+	+	-	+	-	Sebaceous gland adenoma
23.	3/1	0	ML	-	-	-	-	-	Sebaceous gland adenoma
	1/3	1	D	+	-	-	+	+	Meibomian epithelioma
24.	1/3	1	D	+	-	-	-	+	Meibomian epithelioma
25.	1/3	1	D	+	-	-	-	-	Meibomian epithelioma

DISCUSSION

Twenty-eight biopsy samples taken from 25 dogs in total were evaluated in this study. Cocker Spaniel is one of the breeds in which sebaceous tumors are frequently seen,as reflected in our findings. In terms of age, especially with epitheliomas being reported in dogs over 9-10 years of age, our results are consistent with previous literature (Goldschmidt and Goldschmidt, 2017). Although no general gender predisposition was previously stated, it drew attention that the tumors were mostly seen in male animals in our study. While tumor classification was made into 4 groups (sebaceous adenoma, sebaceous ductal adenoma, sebaceous epithelioma, and sebaceous carcinoma) (Goldschmidt and Goldschmidt, 2017), no ductal adenomas were encountered. Same classification was used for Meibomian gland tumors.

Sebaceous adenomas are known to be well-circumscribed nodules and are composed of large amount of typical and uniform sebaceous cells (Goldschmidt and Goldschmidt, 2017). Sebaceous carcinomas are poorly circumscribed nodules composed of large amount of atypical and pleomorphic sebaceous cells. These tumors are distinguishable by the lack of significant numbers of basaloid reserve cells (Goldschmidt and Goldschmidt, 2017). Sebaceous epitheliomas are identified by their predominant population of reserve cells, with small numbers of sebocytes (Goldschmidt and Goldschmidt, 2017). Consistent with previous research, adenomas were well-circumscribed and had a nodular/multi-nodular pattern while carcinomas mainly had a diffuse pattern. In the detailed evaluation of tumor components, the sebaceous-to-basaloid epithelium ratios were examined. The highest ratios were reported for adenomas, epitheliomas and carcinomas with 80% (3:1), 100% (1:3), and 66.7% (2:2) respectively. In a similar study by Costa et al. (2020), the ratios were reported as 55.5% (3:1) for adenoma, 91.6% (1:3) for epithelioma, and 85.7% (3:1) for carcinoma. These findings suggest that carcinomas have a notably higher ratio of sebaceous epithelium.

Necrosis is a well-known criterion to evaluate whether tumors are aggresive and fast-growing (Proskuryakov and Gabai, 2000). Inflammation can also accompany necrosis. In our study both criteria correlated with malignancy of the tumors. The presence of these histopathological criteria in epitheliomas similarly to carcinomas highlights the malignancy potential of epitheliomas as well. .

Regarding pleomorphism, parallel to a similar study (Costa et al, 2020), intermediate pleomorphism (score1 and 2) was prominent in epitheliomas. On the other hand, the presence of only one carcinoma with a pleomorphism score of 3 (the highest) drew our attention.

One of the most important malignancy criteria for tumors is without a doubt the histopathological evaluation of the mitotic activity as a high mitotic index is characteristic of malignant tumors (De Las Mulas et al, 1999; Bertram et al, 2024). In our study, mitotic activity was found to be higher in epitheliomas than in carcinomas. Ki-67 protein comes forth while evaluating the cell division phases (Gerdes et al, 1983; Scholzen and Gerdes, 2000). This protein is only expressed in the growing and dividing phases (G1, S, G2, and M) but not in the resting phase (G0). That is why Ki-67 is a good marker for aggressively proliferating tumor cells (Starborg et al, 1996; Li et al, 2015). Our data suggest that, both epithelioma and carcinoma had moderate ki-67 expression. The conventional criteria for differentiating sebaceous adenoma from epithelioma are not appropriate. Similarly, a study by Keleş et al. (2024) on dogs showed that epitheliomas are potentially malignant. Kim et al. (2024) have also named epithelioma as carcinoma.

CONCLUSION

As a result, prominent changes in subtypes were revealed through detailed histopathological examinations. Furthermore, we suggest that the prognosis of epitheliomas should be examined in more detail due to the fact that they are a frequently observed tumor with low-grade malignancy. Given the findings on low-grade malignancy potential in epitheliomas, future prognostic studies specifically targeting these tumor types would be beneficial.

DECLARATIONS

Ethics Approval

Not applicable.

Conflict of Interest

The authors stated that they have no conflict of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: GYT

Data collection and analysis: GYT, AST, OBD

Drafting of the manuscript: GYT, AST, OBD

Critical review: GYT, AST

Data Availability

Not applicable

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