Cutaneous clear cell adnexal carcinoma in two dogs: cytological and immunohistochemical evaluation

Mehmet Fatih BOZKURT[1,a], Muhammad Nasir BHAYA[1,b,✉], Alper NİŞANCI[2,c]

[1]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Pathology, Afyonkarahisar, Türkiye; [2]Petcode Animal Hospital, Ankara, Türkiye

[a]ORCID: 0000-0002-1669-0988; [b]ORCID: 0000-0001-7696-3039; [c]ORCID: 0000-0002-5771-5280

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✉Corresponding author
dr.muhammadnasir399@gmail.com

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ABSTRACT

In this study, cases of cutaneous clear cell adnexal carcinoma were diagnosed on the right forepaw of a 6-year-old female dog and on the right hind paw of an 8-year-old male dog. On the cytological examination, scattered cell groups were seen on the hemorrhagic background, whose cytoplasmic borders could hardly be distinguished. Although the cells showed marked pleomorphism, but were generally oval, round, or spindle-shaped. Anisokaryosis, karyomegaly, and one or more prominent nucleoli were noted in the nuclei. Pseudoinclusions were found in some cell nuclei. Histologically, it was centrally necrotic, expansive growth consisting of lobular areas in the dermis. The neoplastic cells consisted of oval round-shaped epithelioid cells with clear cytoplasm showing marked anisocytosis, anisorkaryosis and karyomegaly. Nuclei were oval or round in shape with prominent nucleoli. Cystic changes and calcified areas in layers (psammoma bodies) were noted in these areas. Few mitoses were found. In the immunohistochemical examination, tumor cells were positive for vimentin, S-100, MART1 (Melan A), and cytokeratin (MNF116) and negative for glial fibrillary acidic protein (GFAP) and smooth muscle actin (SMA). Based on these findings and results, the tumors were diagnosed as canine clear cell adnexal carcinoma. According to the literature review, this is the first case in which we found psammoma bodies and nuclear pseudo inclusions on microscopic examination of canine cutaneous clear cell adnexal carcinoma.

Canine cutaneous clear cell adnexal carcinoma is one of the rare tumors of dogs and has no differentiation between apocrine, sebaceous and follicular cells (11). First report of this tumor has been published in 1978 with the name of clear cell hidradenocarcinoma in dogs (5). The other reported name of this tumor is follicular stem cell carcinoma (7). Clear cells having vacuolated cytoplasm are the main histopathological findings related to this tumor. Epithelial stem cells of cutaneous region are the main origin for the development of this tumor (7, 11). Other canine cutaneous tumors including balloon cell melanoma, sebaceous carcinoma, clear cell trichoblastoma (12), and clear cell basal carcinoma reveal similarities with the cutaneous clear cell adnexal carcinoma in dogs (3, 4). Schulman et al. (11) have reported this tumor with the name of clear cell adnexal carcinoma for the first time and they proposed that clear cell hidradenocarcinoma and follicular stem cell carcinoma are the same tumors. Mean age of 7 years for the development of this tumor has been reported in dogs. Epithelial or follicular stem cells are the main origin of tumor because the positivity of cytokeratin and vimentin has been reported in different studies (7, 11). The purpose of this study was to evaluate cytological, histopathological and immunohistochemical features of canine cutaneous clear cell adnexal carcinoma and according to literature research, this is the first case in which we found psammoma bodies and nuclear pseudoinclusions on microscopic examination.

Two dogs of 6 and 8 years of old were brought at the Petcode Animal Hospital, Ankara for the examination of swollen masses on right forepaw and right hind paw (Fig 1). Cytological slides were prepared with the help of fine needle aspiration method and Wright stain was performed. After that excisional procedure was performed for the

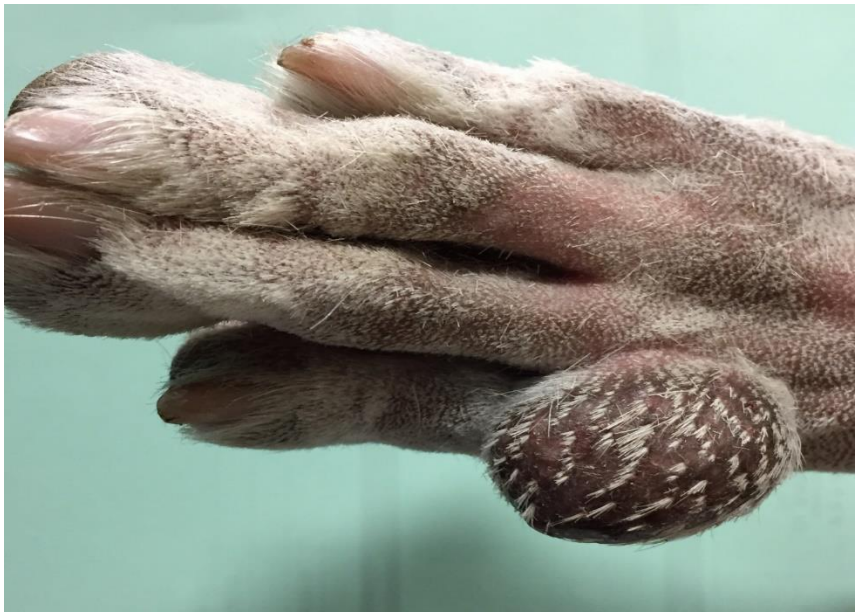

Figure 1. Macroscopic presentation of tumor at right forepaw.

removal of tumor from the right forelimb and right hind limb of dogs. The excisional biopsy was preserved in 10% buffered formalin solution. Later it was sent Pathology laboratory of Afyon Kocatepe University, Afyonkarahisar for the histopathological and immunohistochemical examination.

After cutting and routine processing of tissues, suitable sections of 4µ were taken on slides. Hematoxylin and eosin stain was performed for the histopathological examination. Immunohistochemical evaluation was done for the confirmation of cellular origin tumor. For this staining process, the slides were deparaffinized in xylene. The clearing of tissues was done in graded alcohol solutions. Quenching of endogenous enzymes was performed by treating the tissues with 3% solution of hydrogen peroxide in methanol. After the antigen retrieval primary antibodies of vimentin, S-100, cytokeratin, GFAP and SMA were dropped on the tissues. The detail of primary antibodies for immunohistochemical evaluation is given in Table 1. After overnight incubation secondary antibodies were dropped on the tissues. Special humidity chamber was used for the incubation of slides on room temperature. ABC kit (TA-125-UDX, UltraVision Polyvalent HRP Kit, LabVision / ThermoScientific-USA) was used. Biotinylated IgG was dropped and was incubated at room temperature for 1 hour. Peroxidase conjugated avidin was used and allowed to react for 30 minutes at 37 ° C. Slides were washed with buffer solution and tissues were treated with red colored AEC (TA-060-HA, AEC Substrate System, LabVision / ThermoScientific-US) peroxidase substrate. After completion of reaction, the slides were taken into distilled water and counter stained with Mayer's hematoxylin. Slides were covered with coverslips using aqueous adhesive medium and examined under a light microscope (Zeiss Axio Lab.A1 Microscope - AxioCam ICc 5 Camera).

Table 1. The detail of antibodies used in immunohistochemical analysis of canine clear cell adnexal carcinoma.

Primary antibody	Detail of primary antibodies	Species
Cytokeratin (MNF116)	Santa Cruz, SC-58830	Mouse
Vimentin	Abcam, 3B4, ab28028	Mouse
S100	Thermo Fisher Scientific, RB-1805-A	Rabbit
MART1 (Melan A) (Ab-4)	Thermo Scientific, MS-799-P1	Mouse
SMA	Dako M0851	Mouse
GFAP	Thermo Scientific, RB-087	Rabbit

Cytological examination revealed scattered cell groups with clear cytoplasm borders on the hemorrhagic background. Although the cells showed marked pleomorphism, but were generally oval, round or spindle-shaped. Anisokaryosis, karyomegaly and one or more prominent nucleoli were noted in the nuclei. Pseudoinclusions were found in some cell nuclei (Fig 2 A-B). Histopathological examination showed that tumor was centrally necrotic, expansive growth consisting of lobular areas in the dermis. The neoplasic cells consisted of oval round shaped epithelioid cells with clear cytoplasm showing marked anisocytosis and anisorkaryosis. Their nuclei were oval or round in shape with prominent nucleoli. Cystic changes and calcified areas in layers (psammoma bodies) were noted in the areas. Few mitoses were found (Fig 2 C-D).

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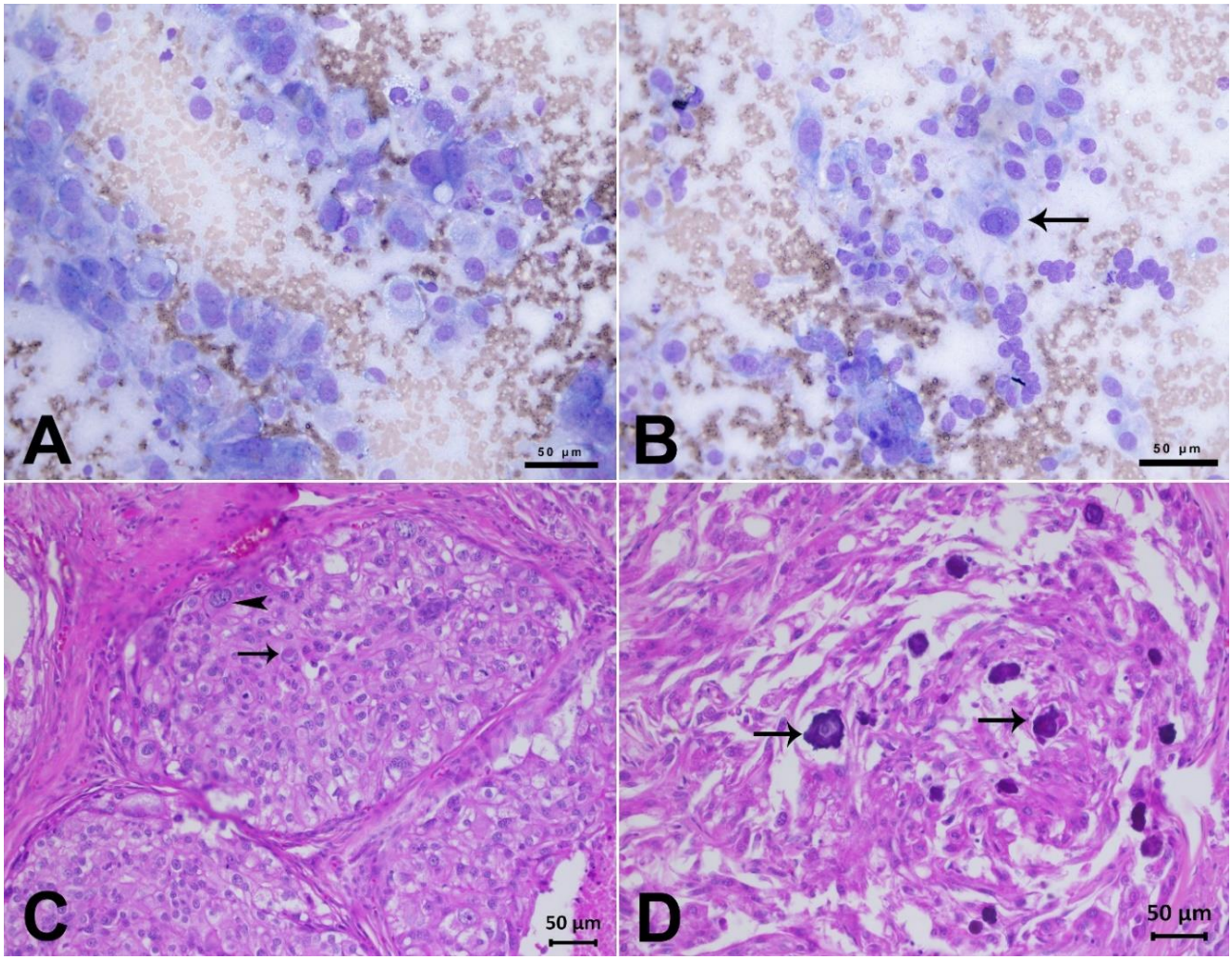

Figure 2. Cytological (A-B) and histopathological (C-D) images of the case (Scale bars=50 µm). **A-B.** Prominent and multi-nucleated oval nucleated, oval or round cytoplasm showing anisocytosis, anisokaryosis, karyomegaly and pseudoinclusion body (shown with arrow in B) (Wright's Stain). **C.** The neoplasic cells consisted of oval round shaped epithelioid cells with clear cytoplasm showing marked anisocytosis, anisorkaryosis , karyomegaly (arrow head) and psuedoinclusion (arrow) in lobular areas (H&E). **D.** some mineralization (psammoma bodies, shown with arrows) in neoplastic areas (H&E).

For immunohistochemical examination vimentin, cytokeratin, MART1, S-100, GFAP and SMA markers were used. Multifocal positivity with vimentin (Fig 3A), strong positivity especially of basal cells with cytokeratin (Fig 3B), diffuse positivity with MART1 (Fig 3C), and nuclear and cytoplasmic positivity with S-100 (Fig 3D) were evaluated. GFAP and SMA revealed no positivity.

In this study we have evaluated the cytological, histopathological and immunohistochemical features of canine cutaneous clear cell adnexal carcinoma. Cellular pleomorphism, loosely arranged oval to polygonal neoplastic cells with cytoplasmic projections and pink colored inclusions have been reported in previous cytological study (9). The criteria for malignancy was significant anisocytosis, anisokaryosis, pleomorphism, multinucleation, karyomegaly, and atypical mitotic figures (9). Cytological results of this study were also similar to the previously reported study. The presence of cytoplasmic pseudoinclusions has been evaluated during cytological examination in this report that was not found in previous studies. Pleomorphic neoplastic cells with clear cytoplasm, multinucleation of oval to polygonal cells has been reported in different studies (9, 11, 13). Histopathological results of this study revealed similar findings like previous studies. The difference from the previous studies in histopathological findings was the presence of psammoma bodies in this tumor. For the confirmation of diagnosis of canine cutaneous clear cell adnexal carcinoma, staining of different markers including cytokeratin, vimentin, Melan A, S-100 and smooth muscle actin has been reported. The positive results of vimentin, cytokeratin, S-100 and Melan A and negative results of smooth muscle actin have been evaluated in these studies (9, 11, 13). The immunohistochemical results of this study were found similar to the results of previous studies.

The differential diagnosis of canine cutaneous clear cell adnexal carcinoma with the other cutaneous tumors is really important. Cytokeratin could not be positive for the

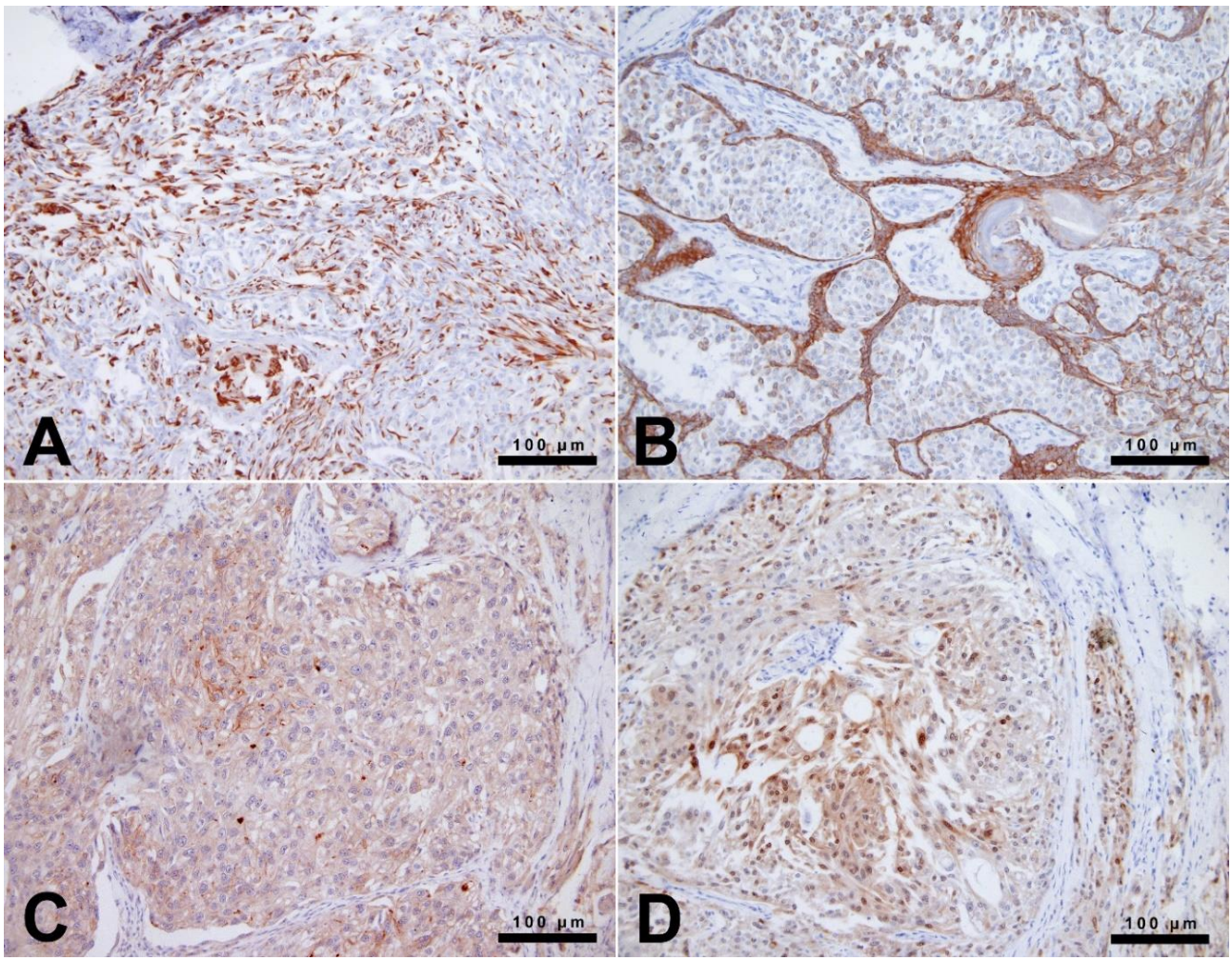

Figure 3. Immunohistochemical images of the case (Scale bar=100 µm). **A.** Multifocal positivity with vimentin. **B.** Strong positivity especially of basal cells with cytokeratin. **C.** Diffuse positivity with MART1. **D.** Nuclear and cytoplasmic positivity with S-100.

balloon cell melanoma and also it shows junctional activities that were not found in this report (11, 13). Positivity of Melan A and lack of sebaceous cells differentiation revealed that this is not a sebaceous carcinoma (4, 11). Epidermal contiguity has been found in clear cell basal carcinoma and it was not found in this report (3, 4). The negative result of smooth muscle actin is a consistent result with the previous studies (7, 11, 13). Liposarcoma has special features and it only shows the positivity of cytokeratin (11). Immunohistochemical results of this study were consistent with the correct diagnosis of cutaneous clear cell carcinoma in dogs. Psammoma bodies have normally been reported in different studies of meningioma in dogs (6, 8, 14) and cats (10). The positivity of vimentin and S-100 in meningioma (1, 2, 8) like clear cell adnexal carcinoma has also been reported. The presence of psammoma bodies in meningioma and clear cell adnexal carcinoma may have correlation. Future investigations have required to find out the correlation between the cells of origin of tumors.

Canine cutaneous clear cell adnexal carcinoma is a rare tumor and this study evaluated the cytological, histopathological and immunohistochemical features of this tumor. The presence of pseudoinclusions and psammoma bodies during microscopical examination were the interesting findings that have not been reported in previous studies.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

Diagnose, Investigation, Writing-Reviewing and Editing are made by MFB and MNB. Clinical evulation contributed by AN.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

There was no need of any ethical report for this study.

Animal Welfare

Not applicable.

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