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Case Report

Desmoplastic Small Round-Cell Tumor in a Dog [1]

Mehmet Eray ALÇIĞIR [1,a] Tuncer KUTLU [2,B] Ekrem Çağatay ÇOLAKOĞLU [3,c] Ceyda DİNÇ [3]

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[1] Kırıkkale University, Faculty of Veterinary Medicine, Department of Pathology, TR-71451 Yahsihan, Kırıkkale - TURKEY
[2] Hatay Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Pathology, TR-31060 Alahan, Antakya/Hatay - TURKEY
[3] Ankara University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-06110, Diskapı, Ankara - TURKEY
[a] ORCID: 0000-0002-5165-5854; [b] ORCID: 0000-0002-8771-1256; [c] ORCID: 0000-0003-2789-035X

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Abstract

A 13-year-old, neutered female Husky dog was brought to the clinic with the complaints of anorexia, vomiting, abdominal distension and respiratory distress. It was suddenly died during intervention. At the necropsy, a mass was seen in the abdominal cavity. The mass had 16x15x9 cm in diameters. Histological examination revealed clusters of cells with slightly eosinophilic and scanty cytoplasm, small round hyperchromatic nuclei, and inconspicuous nucleoli encompassed by hypocellular extensive desmoplastic connective tissue stroma comprising few spindle-shaped connective tissue cells. In immunohistochemical examination the cytoplasms of tumour cells were detected to be mildly positive by nestin. The tumour cells were negative for α-SMA (Alpha-Smooth Muscle Actin), vimentin and pancytokeratin, but stromal cells were positive for α-SMA and vimentin. Despite the presence of partially incompatible immunohistochemical findings, the tumor in this case was diagnosed as desmoplastic small round-cell tumor because its aggressiveness, localization, and histopathology was similar to that observed for this tumor in humans. Previously this tumor has not been identified in animals.

Keywords: *Clinicopathology, Desmoplastic tumor, Dog, Immunohistochemistry*

Bir Köpekte Küçük Yuvarlak Hücreli Dezmoplastik Tümör

Öz

Husky ırkı, 13 yaşlı, kısırlaştırılmış dişi bir köpek, anoreksi, kusma, karın şişliği ve solunum sıkışması şikayetiyle kliniğe getirildi. Hayvana müdahele sırasında aniden öldü. Nekropside karın boşluğunda 16x15x9 cm boyutlarında bir kitleyle karşılaşıldı. Histolojik incelemede hiposelüler, az sayıda mekik şekilli bağ doku hücrelerinden oluşan geniş desmoplastik bağ doku stromasıyla adacıklara ayrılan; hafif eozinofilik dar sitoplazmalı, küçük yuvarlak-oval hiperkromatik çekirdekli, çekirdecikleri fark edilmeyen hücre kümeleri gözlendi. İmmunohistokimyal incelemede tümör hücre sitoplazmalarının nestin yönünden hafif pozitif olduğu saptandı. Tümör hücreleri α-SMA (Alpha-Smooth Muscle Actin), vimentin ve pansitokeratin negatif olup stroma α-SMA ve vimentin pozitifti. İmmunohistokimyasal yönden kısmen farklı bulgular göstermekle beraber gerek lokalizasyonu gerekse histomorfolojik açıdan insanlardakine benzerliklerinden dolayı bu tümöre desmoplastik küçük yuvarlak hücreli tümör teşhisi konmuştur. Daha önce bu tümör hayvanlarda tanımlanmamıştır.

Anahtar sözcükler: *Dezmoplastik tümör, Klinikopatoloji, İmmunohistokimya, Köpek*

INTRODUCTION

Desmoplastic small round-cell tumor was first described in humans by Gerald and Rosai in 1989 [1]. It is a very aggressive and rare tumor and is usually reported in young male adults. It typically affects the peritoneum (88%) [2,3]. While its etiopathogenesis is still unknown, it is histologically composed of nests and large clusters of small round cells with scanty cytoplasm and hyperchromatic nuclei separated by fibrosclerotic desmoplastic stroma [2,4]. Immunohistochemistry revealed that the tumor was positive for epithelial (cytokeratin [95%] and Epithelial Membrane Antigen [EMA] positivity), mesenchymal (desmin and vimentin positivity [81%]), and neural (NSE, CD57 and synaptophysin positivity) markers [2,4,5]. However, the tumor was also positive for vimentin and cytokeratin (AE1/AE3) [6] markers and negative for α-SMA [4]. We aimed to examine morphopathological and immunohistochemical

İletişim (Correspondence)
+90 326 2455313
tuncerkutlu83@gmail.com

characteristics of such a tumor present in a dog; the dog's tumor characteristics were consistent with those of this rare human tumor in terms of localization, histopathology, and aggressiveness.

CASE HISTORY

A 24 kg, 13-year-old, neutered female Husky breed dog referred to Veterinary Teaching Hospital with a history of anorexia, vomiting, abdominal distention and respiratory distress. The dog was receiving a medication for gastritis at the time of referral investigation. Physical examination revealed severe abdominal distress and pain in right abdominal region. Poor general health status, mucosal pallor and increased capillary refill time (>3s) were also remarkable. The dog was hypothermic (36.9°C) and tachypneic (respiratory rate = 40-53 breaths per minute) with increased respiratory effort. Femoral arterial pulses were slightly weak. It could not be possible to auscultate the heart rate because of the severe tachypnea. A serum biochemistry panel showed hypoalbuminemia, hypoproteinemia and increased blood levels of ALP and GGT *(Table 1)*.

Necropsy revealed a 16×15×9 cm-sized, round shaped, elastic, lobular mass lesion with a grayish–red color between the liver, spleen, and bowel; the lesion was adhered to the dorsal peritoneum of the abdomen and cranial pole of the right kidney *(Fig. 1)*.

Tissue samples were fixed in 10% buffered formalin solution. After fixation, the tissues were dehydrated to enable embedding with paraffin. The tissues were dehydrated gently by immersion in increasing concentrations of alcohol (70%, 80%, 96%, 100%). The dehydrating agent was then cleared by incubation in xylene prior to paraffin embedding. Next, 5 µm thick sections were cut from paraffin-embedded blocks, deparaffinized in xylol, and stained with Harris' Hematoxylin and Eosin after being passed through a series of 100%, 96%, 80%, and 70% alcohol treatments. After Harris' hematoxylin and eosin (H&E) staining, the sections were examined by light microscopy. Histological examination revealed clusters of cells with slightly eosinophilic and scanty cytoplasm, small round hyperchromatic nuclei, and inconspicuous nucleoli encompassed by hypocellular extensive desmoplastic connective tissue stroma comprising few spindle-shaped connective tissue cells *(Fig. 2a, b)*. High numbers of mitotic figures were encountered in all microscopic fields *(Fig. 2c)*. Moreover, foci of bleeding and necrosis were observed in some fields *(Fig. 2d)*.

Immunohistochemistry was carried out using indirect immunoperoxidase method (ABC-P) method using primary antibodies against cytokeratin, α-SMA, vimentin, and nestin. Primary antibody dilution rate, incubation duration, incubation temperature, antigen retrieval, and endogenous biotin block are shown in *Table 2*. After deparaffinization and dehydration, peroxidase activity was blocked for 30 min by peroxidase blocking reagent (Novocastra Peroxidase Detection Systems, Ready to use). A SensiTek HRP (ScyTec Laboratories, Super block LOT: 24062; Biotinylated antibody LOT: 24205; HRP LOT: 24242®) kit was used in accordance with the manufacturer's instructions. Either heat or trypsin was used for antigen retrieval, and 3,3'-diaminobenzidine (DAB) (DAB-Substrate Kit, Invitrogen, 896320A®) was used as a chromogen. The heated sections were applied egg white and milk powder for the blocking of endogenous

Table 1. *Results of routine blood work in the dog*

Complete Blood Count	**Results**	**Reference Ranges**
WBC (10^9/L)	15.8	6-17
LYM (10^9/L)	2.4	0.9-5
MONO (10^9/L)	1.1	0.3-2.5
NEUT(10^9/L)	10.4	3.5-12
EOS (10^9/L)	1.9	0.1-19
LYM %	15.3	12-30
MON %	7.0	2-13
NEU %	66.1	35-70
EOS %	11.4	0.1-19
RBC	6.17	5.5-8.5
HGB	14.8	12-18
HCT	35	37-55
MCV	56.7	60-72
MCH	24	19.5-25.5
MCHC	42.3	32-38.5
RDWa	33.9	35-53
RDW %	18.3	12-17.5
PLT (10^9/l)	433	200-500
MPV (fl)	7.6	5.5-10.5
Serum Biochemistry		
Glucose (mg/dL)	110.5	65-118
Urea (mg/dL)	12.5	15-59.9
Creatinine (mg/dL)	0.62	0.5-1.5
Total Protein (g/dL)	4.6	5.4-7.1
Albumin (g/dL)	2.7	3.1-4.0
Total Bilirubin (mg/dL)	0.13	0.1-0.3
Direct Bilirubin (mg/dL)	0.08	-
ALP (IU/L)	169.9	20-156
ALT (IU/L)	38.7	21-102
AST (IU/L)	52.6	23-66
GGT (IU/L)	145	6-28
Creatine Kinase (IU/L)	154	<200
Na (mmol/L)	133	140-154
K (mmol/L)	5	3.8-5.6

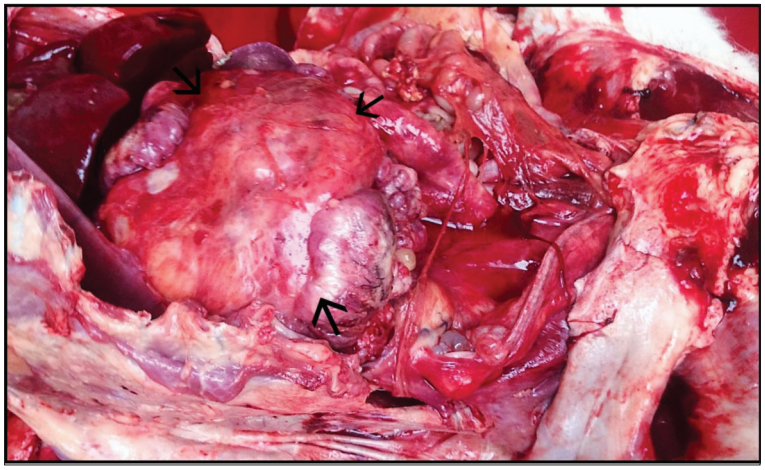

Fig 1. Macroscopical view of desmoplastic tumor *(arrows)*

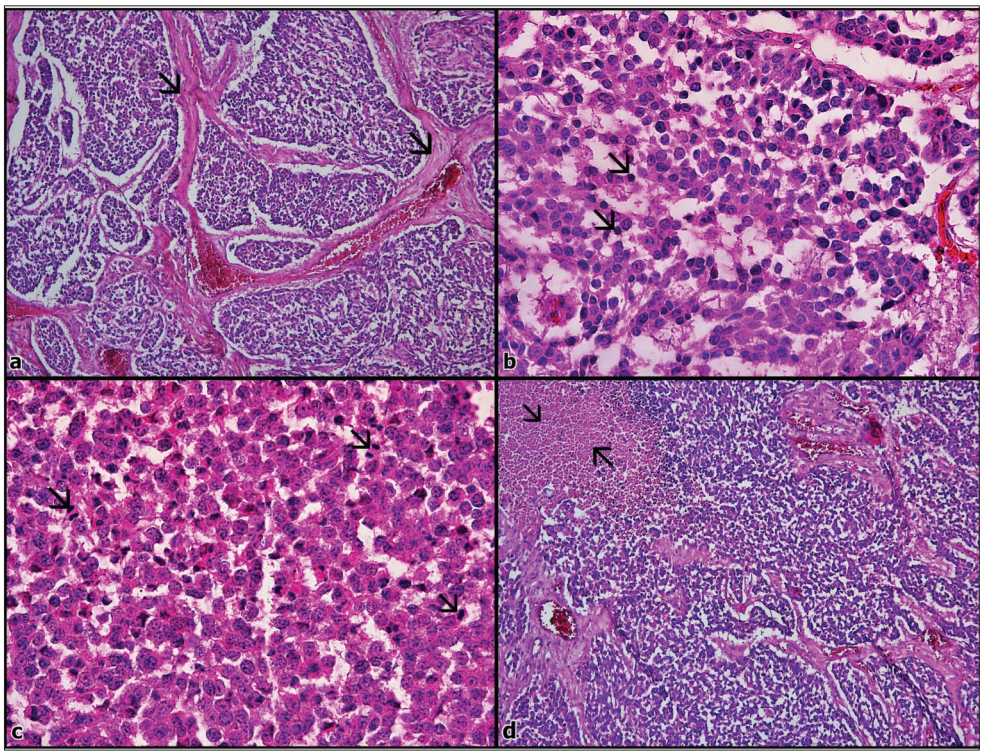

Fig 2. Histopathological findings **a-** Fibrosclerotical desmoplastic stroma forming cell islands *(arrows)*, x100, HE. **b-** Tumor cells having small round-oval hyperchromatic nuclei *(arrows)*, x400, HE. **c-** Numerous mitotic figures (arrows), x400, HE. **d-** Extensive areas of necrosis *(arrows)*, x100, HE

Table 2. *The dilution rate of the primary antibodies, the duration of the incubation period, the incubation temperature, antigen retrieval and endogenous biotin block*

Primary Antibodies	Antigen Retrieval	Endogenous Biotin Block	The Dilution Rate of the Primary Antibodies/Duration/ Temparature	Chromogen
Nestin (Acris, AP07829PU-N)®	Citrate Buffer (pH 6.0)+0.1% Tween; 700 watt, 3x5 min/heat	Egg white and milk powder (7)	1/100 PBS Overnight; +4°C	DAB (Invitrogen, 896320A)®
Vimentin antibody (Dako, Vim3B4)®	5 min 0.1% trypsin 37°C	-	1:100 PBS 75 min; 37°C	DAB (Invitrogen, 896320A)®
α-SMA antibody (Sigma, 120M4768)	10 min , 0.1% trypsin 37°C	-	1:200 PBS 45 min; 37°C	DAB (Invitrogen, 896320A)®
Pancytokeratin antibody (AE1/AE3+5D3) (Abcam, ab86734)®	5 min, 0.1% trypsin 37°C	-	1:100 PBS 1 h; 37°C	DAB (Invitrogen, 896320A)®

biotin [7]. Immunohistochemical examination of stroma cells revealed positive staining for vimentin and α-SMA *(Fig. 3a)*. Tumor cells revealed negative staining for α-SMA, pan-cytokeratin *(Fig. 3b)* and vimentin *(Fig. 3c)*. The cytoplasm of tumor cells revealed slightly positive staining for nestin *(Fig. 3d)*.

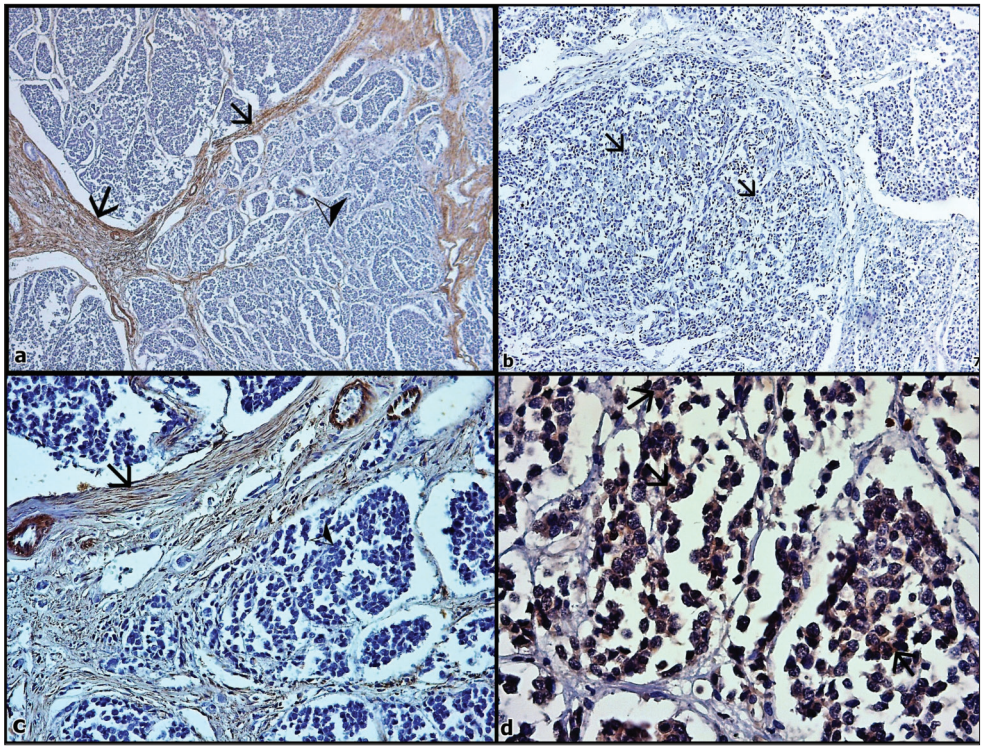

Fig 3. Immunohistochemical findings **a-** Tumor cells were negative with alpha SMA (arrow head) but stromal cells were positive *(arrows),* x50, ABC-P. **b-** Tumor cells were negative pancytokeratin *(arrow),* x100, ABC-P. **c-** Tumor cells were negative with vimentin *(arrow head)* but stromal cells were positive (arrow), x200, ABC-P. **d-** Cytoplasms of tumor cells were detected to be mildly positive by nestin *(arrow),* x400, ABC-P

DISCUSSION

Involvement of the peritoneal surfaces and aggressiveness are the prominent characteristics of desmoplastic small round-cell tumors [2,3]. Tumor evaluation in the present case also indicated desmoplastic small round-cell tumor owing to the involvement of the peritoneum and the tumor having reached a large size.

Histopathological examination of the tumor in the present case revealed presence of cells with slightly eosinophilic and scanty cytoplasm, small round hyperchromatic nuclei, and inconspicuous nucleoli forming islands through the connective tissue stroma; these microscopic features were similar to those observed for desmoplastic small round-cell tumors [2,4].

Etiopatogenesis of desmoplastic small round-cell tumor is still unknown and diagnosis can be achieved only by immunohistochemistry and sitogenetic studies [4]. Although immunohistochemical studies reported positive staining for epithelial, mesenchymal, and neural markers in desmoplastic small round-cell tumors [2,4,5], tumor cells in this case tested negative for α-SMA, vimentin, and pancytokeratin and only the tumor cytoplasm revealed positive staining for nestin. Xie and Shen [6] reported that 87% of these tumors revealed positive staining for cytokeratin (AE1/AE3); however, the present case revealed negative staining. Koniari et al.[4] reported negative staining, for α-SMA, a finding consistent with that observed in the current case.

Despite the presence of partially incompatible immunohistochemical findings, the tumor in this case was diagnosed as desmoplastic small round-cell tumor because its aggressiveness, localization, and histopathology was similar to that observed for this tumor in humans. Moreover, this is the first report of desmoplastic small round-cell tumor diagnosed in an animal.

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