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Research Article

CD3, CD79αcy, Vimentin, α-Smooth Muscle Actin, S100 and Cytokeratin 5/6 Expression in Immunohistochemical Characterization of Dogs with Transmissible Venereal Tumors

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Abstract

Transmissible Venereal Tumor (TVT) is a highly contagious round cell tumor and its origin is unknown. In our study, we aimed to evaluate CD3, CD79αcy, vimentin, α-smooth muscle actin, S100, and cytokeratin 5/6 expressions immunohistochemically in order to reveal the cellular characterization of TVT. The material of this study consisted of ten dog tissue sections, six females and four males diagnosed with TVT. Tissue samples from dogs were fixed in a 10% buffered formaldehyde solution. After routine tissue procedures follow-up, Hematoxylin & Eosin stain was applied to the sections and investigated under a light microscope. The smear prepared from the tumor was examined with the Diff-Quick method for cytologically. Immunohistochemical staining was performed on the tissues using the avidin-biotin immune peroxidase complex method. Based on macroscopic, histopathological and cytological findings, we decided that the cases were TVT. All cases were negative for all markers except vimentin expression in round tumoral cells. The negative immunoreactivity of CD3, CD79αcy, α-smooth muscle actin, and cytokeratin 5/6 in tumor cells suggests that the origin of the tumor is not lymphocytic, smooth muscle cells or epithelial. Only the option of histiocytic origin remains.

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Köpeklerin Bulaşıcı Veneral Tümörlerinin İmmunohistokimyasal Karakterizasyonunda CD3, CD79αcy, Vimentin, α-Smooth Muscle Actin, S100 ve Sitokeratin 5/6 Ekspresyonları

Özet

Bulaşıcı Veneral Tümör (BVT) oldukça bulaşıcı, yuvarlak hücreli bir tümördür ve tümörün kökeni bilinmemektedir. Bu çalışmamızda BVT'nin hücresel karakterizasyonu ortaya koymak amacıyla CD3, CD79αcy, vimentin, α-düz kas aktin, S100 ve sitokeratin 5/6 ekspresyonlarını immunohistokimyasal olarak değerlendirmeyi amaçladık. Bu çalışmanın materyalini altısı dişi ve dördü erkek olmak üzere toplamda on adet BVT teşhisi konulmuş kesit oluşturdu. Köpeklerden alınan doku kesitleri %10'luk tamponlu formaldehit solüsyonunda tespit edildi. Rutin doku işlemleri ardından kesitlere Hematoksilen & Eozin boyası uygulandı. Kesitler ışık mikroskobu altında değerlendirildi. Sitolojik incelemeler için kitlelerden alınan smearlere Diff-Quick metodu uygulandı. İmmunohistokimyasal boyama olarak avidin biotin immune peroksidaz kompleks yöntemi uygulandı. Makrosobik, histopatolojik ve sitolojik bulgular temelinde vakalara TVT teşhisi konuldu. Tüm vakalar yuvarlak tümör hücrelerinde vimentin ekspresyonu dışında tüm belirteçler için negatifti. CD3, CD79αcy, S100, sitokeratin 5/6 and α-düz kas aktin immunoreaktivitesi açısından negatiflik tümörün kökeninin lenfositer, düz kas hücresi ya da epitelyal kökenli olmadığını düşündürmektedir. Geriye kalan tek seçenek tümörün histiyositer kökenli olabileceği yönündedir.

Anahtar sözcükler: *Bulaşıcı veneral tümör, immunfenotip, köpek*

Introduction

Transmissible Venereal Tumor (TVT), also known as Sticker's tumor, transmissible lenfosarcoma, venereal granuloma, infectious sarcoma, is a benign tumor of dogs (Pir Yağcı & Kalender 2008). TVT is an immune related highly contagious round cell tumor and its origin is unknown (Park et al. 2006; Farjanikish 2017). This tumor, especially seen in sexually active and young female dogs, is transmitted among dogs through mating (Çeşme et al. 2015). Transmission can also occur via behavioral social expressions such as biting, sniffing or licking (Sudjaidee et al. 2015).

The tumor is mostly seen in external genital organs and less frequently in internal genital organs (Oruç et al. 2011). In female dogs, the localization of the tumor is usually in the posterior aspect of the vagina, where it usually joins the vestibule. Sometimes it surrounds the urethral orifice and can come out of the vulva. The tumor is located especially on the penis in male dogs, it can sometimes be observed on the prepuce (Akkoc et al. 2017). Although this tumor has been detected in many regions worldwide, it is more common in dogs that show free movement populations in tropical and subtropical countries (Özenç et al. 2016; Oguş & Özmen 2018). There are opinions of different researchers that the tumor may be of different cell origin, such as lymphocyte, histiocyte, and reticulum cells (Çizmeci et al. 2012). It is thought that it may be associated with a viral agent (Uçar 2016).

CD3 is a surface antigen specific for T lymphocytes, indicating the T cell distribution in tissues. Normally, CD3 has both inducer suppressive cytotoxic effects in mature peripheral T lymphocytes. In immunohistochemical analysis, it interacts with the epsilon chain and reacts in T cells (Beverley 1981; Mukaratirwa & Gruys 2003). CD79 is a transmembrane protein that complexes with B cell receptors and consists of two chains, CD79A and CD79B. CD79 antigen B forms part of the lymphocyte receptor complex and plays a role in mediating the transport of IgM to the cell surface. CD79 is not found in myeloid and T cells. Therefore, it is stained to identify B cell neoplasms (Astsaturov 1996; Milner 1996; Pérez et al. 1998). Vimentin is one of the 5 main types of cytoplasmic intermediate filaments and is the first type 3 intermediate filament to be expressed in all mesenchymal embryonic cells. Endothelial cells are found in mesenchymal origin cells such as fibroblast and vascular smooth muscle cells. They also constitute the important skeletal structure of these cells (Rosai 1996; Marchal et al. 1997; Tosun 2009). Vimentin is also expressed by TVT cells (Araújo et al. 2012). Cytokeratin 5 / 6 is a cytoplasmic intermediate filament produced mainly in keratinized and non-keratinized epithelium, prostate basal cells, salivary gland and breast cells (David et al. 2003; Akpınar, 2010). The S100 protein is used to distinguish the infectious venereal tumor of dogs from amelanotic melanoma. While amelanotic melanomas express the S100 protein, the infectious venereal tumor of dogs does not express this protein (Sandusky et al. 1987; Mozos et al. 1996). A-smooth muscle actin, encoded by the ACTA2 gene, is an isoform of vascular smooth muscle actin. Typically expressed in vascular smooth muscle cells that contribute to vascular motility and contraction (Yuan, 2015). This antibody has specificity to muscle cells (Mukaratirwa and Gruys, 2003).

In this study, we aimed to evaluate CD3, CD79 αcy, vimentin, α-smooth muscle actin, S100, and cytokeratin 5/6 expressions immunohistochemically in order to reveal the cellular characterization of Kangal and Kangal hybrid dogs with TVT.

Materials and methods

Animals and ethics committee

The material of this study consisted of ten dog tissue sections, six females and four males diagnosed with TVT, with histopathological findings brought to our department between 2010 and 2020. Metastatic cases were not found in clinical and radiographic examinations. When the routine treatments of the patients were completed in the clinic, they were discharged. Sex, breed, age, location of the tumor, stage, and metastasis information of the animals were given in Table 1. The ethics committee report of this study was obtained from Kafkas University Animal Experiments Local Ethics Committee.

Histopathological, Diff-Quick and immunohistochemical methods

Tissue samples from dogs were fixed in a 10% buffered formaldehyde solution. After routine tissue procedures follow-up, 5 µm thick sections were taken from paraffin blocks. To reveal histopathological changes, Hematoxylin & Eosin (H&E) stain was applied to the sections. Sections were investigated under a light microscope (Olympus Bx53) and photographed with Cell ^P Program (Olympus Soft Imaging Solutions GmbH, 3,4). The smear taken as sliding over the mass was used with the Diff-Quick method for cytological examinations. Diff-Quick staining, a modified version of Giemsa staining, is one of the fastest methods. This method used in our clinical routine is easy and reliable to apply. Swaps and smear samples were taken from the masses in the vagina or penis. Spread the sample onto the slide and dry it. For Diff-Quick staining, methanol is kept for 20 seconds in fixative, 20 seconds in orange solution (solution I), 20 seconds in purple solution (solution II), and 10 seconds in distilled water. It was then kept in ethanol (95%) for 10 seconds, dried in air, and kept in xylol for 15 seconds, then sealed with entellan (Dinç 2005). Immunohistochemical staining was performed on the tissues using the avidin-biotin immune peroxidase complex method. For immunohistochemical staining, sections of 4 µm thick from paraffin blocks were rehydrated.

Table 1. Sex, breed, age, location of the tumor, stage and metastasis information of the animals

Case No	Sex	Breed	Age	Location	Stage	Metastasis
Case 1	Female	Kangal	3	Vagina	Progression	-
Case 2	Female	Kangal	5	Vagina	Progression	-
Case 3	Male	Kangal	5	Penis	Progression	-
Case 4	Female	Kangal hybrid	5	Vulva	Progression	-
Case 5	Male	Kangal	4	Prepuce	Progression	-
Case 6	Female	Kangal hybrid	5	Vagina	Progression	-
Case 7	Male	Kangal	3	Penis	Progression	-
Case 8	Male	Kangal hybrid	4	Penis	Progression	-
Case 9	Female	Kangal	5	Vulva	Progression	-
Case 10	Female	Kangal	4	Vagina	Progression	-

To prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution for 15 minutes. The microwave method was applied to the sections to reveal the antigenic receptors (Citrate Buffer Solution pH 6 for 25 min). In order to prevent nonspecific staining, the sections were incubated for 30 minutes with non-immune serum (Genemed Biotechnologies REF 54-0003). Following treatment with Phosphate Buffered Salt Solution (PBS) with different antibodies; (CD3: Thermo Fisher Scientific, MA1-90582, ready to use), (CD79αcy: Thermo Fisher Scientific, SP7, Dilution Ratio 1:400), (Vimentin: Thermo Fisher Scientific, SP-20, ready to use), (α-smooth muscle actin Ab-1: Thermo Fisher Scientific, 1A4, ready to use), (S100: Thermo Fisher Scientific, 4C4.9, ready to use) and (Cytokeratin 5/6: Thermo Fisher Scientific, D5/16 B4, ready to use) were incubated for overnight. The sections were washed 3 times in PBS solution for 5 minutes, and the biotinylated secondary antibody (Genemed Biotechnologies REF 54-0003) was applied to them at room temperature for 30 minutes. After washing in PBS (3-5 min), all sections were incubated with peroxidase-bound Strep Avidin (Genemed Biotechnologies REF 54-0003) for 30 minutes. A solution of 3.3-diaminobenzidine tetrahydrochloride (DAB) (Genemed Biotechnologies REF 10-0048) was used as color revealing substrate. The sections were stained with Mayer Hematoxylin and coated with immune mount. While primary antibodies were not applied to negative control sections, diluted normal serum was applied to these sections. The sections prepared after the covering were examined under a light microscope (Olympos Bx53) and photographed via the Cell^P program (Olympos Soft Imaging Solutions Gmbh, 3,4).

Results

Macroscopical results

Female dogs brought to our clinic with complaints of bloody discharge in the vulva, external appearance of the mass and frequent licking of the area, and male dogs with swelling in the penis / prepuce area, sometimes with serosanguinous or hemorrhage.

When examined clinically, masses have cauliflower appearance, nodular structure, ranging from 5 mm to 15 mm, with a crunchy consistency, ulcers with ulcers and/or inflammation, sometimes with bleeding were determined. In females, it was determined that the masses were located in the vagina and vulva, whereas in males, the masses were located in the caudal part of the penis. None of the patients brought to our clinic has a history of chemotherapy before. Besides, we learned from the animal owners that they encountered such a situation for the first time in the anamnesis information received. (Figure 1).

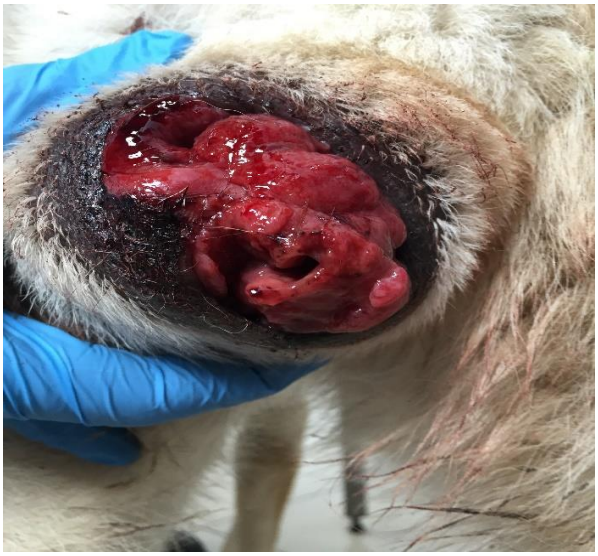

Figure 1. Macroscopic appearance of the tumor

Histopathological Results

In histopathological examination, we observed that tumor cells as confluent layers or solid masses. We detected tumor cells with hyperchromatic nuclei surrounded by thin stroma. The nuclei of the tumor cells were eccentrically located. In addition, we detected the presence of a large number of mitotic figures. When the developmental stages of the tumors were examined, we determined that all of them were in the stage of progression. We demonstrated mononuclear cell infiltrations, mostly lymphocytes, among tumor cells. Necrosis and hemorrhage in some cases were other remarkable findings (Figure 2).

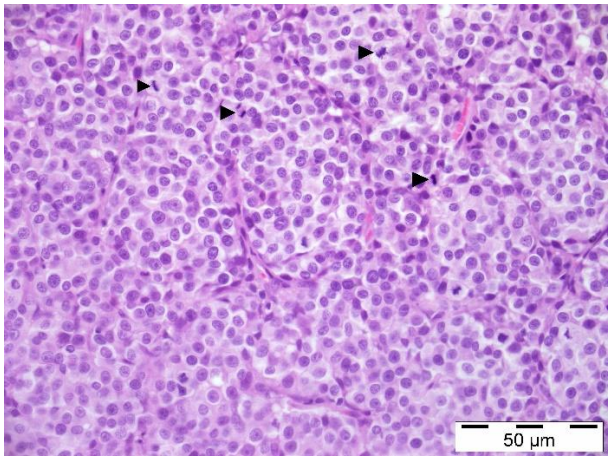

Figure 2. Tumor cells surrounded by thin stroma, and mitotic figures (arrowheads), H&E, Bar=50 µm.

Cytological Results

In cytological examinations, we detected tumor cells with large and vacuolar cytoplasm, hyperchromatic and prominent nuclei. We also found that the nucleus / cytoplasm ratio increased in favor of the nucleus. The increase in the nucleoli number was one of the other cytological findings. (Figure 3).

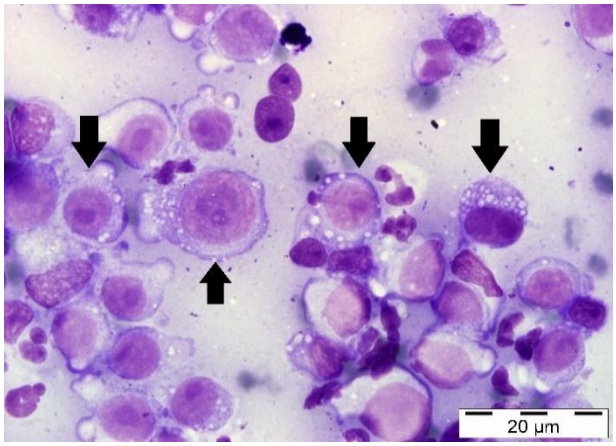

Figure 3. Cytological appearance of TVT cells (arrows), Diff-Quick, Bar=20 µm.

Immunohistochemical Results

CD3 immunoreaction was strongly with intra- and peritumoral infiltrated lymphocytes. We did not detect CD3 immunoreactivity in TVT tumoral cells (Figure 4). We observed that CD79αcy expression in intra-tumoural infiltrating lymphocytes and plasma cells very strong (Figure 5). We detected vimentin expression in round tumoral cells. The cytoplasm of cells was stained densely (Figure 6). α-smooth muscle actin expression was observed in stromal cells and muscle cells from blood vessel walls (Figure 7). All TVT cases were negative for S100 and cytokeratin 5/6 immunoreactivity (Table 2).

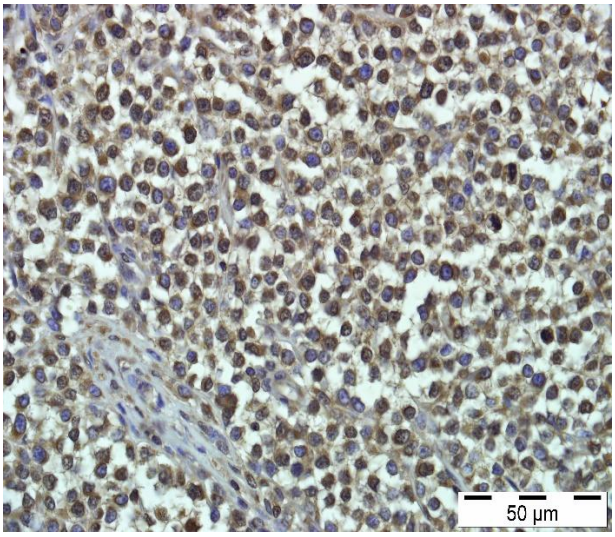

Figure 4. CD3 expression in intra-tumoural infiltrating lymphocytes, IHC, Bar=50 µm.

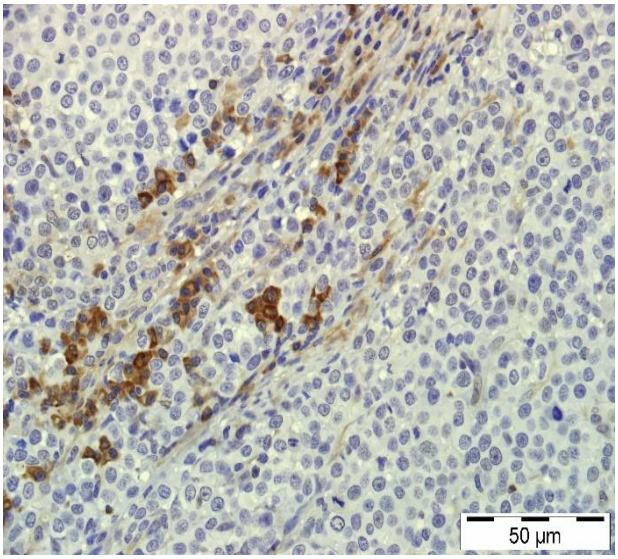

Figure 5. CD79αcy+ diffuse cytoplasmic expression in intra-tumoural infiltrating lymphocytes, IHC, Bar=50 µm

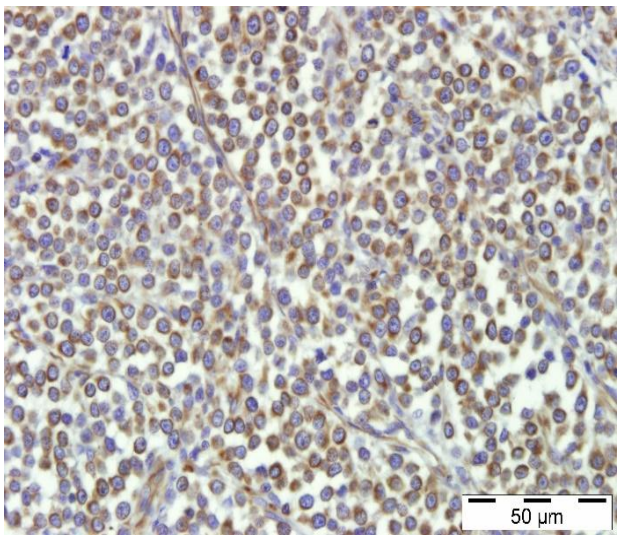

Figure 6. Vimentin expressions in round tumoral cells, IHC, Bar=50 µm.

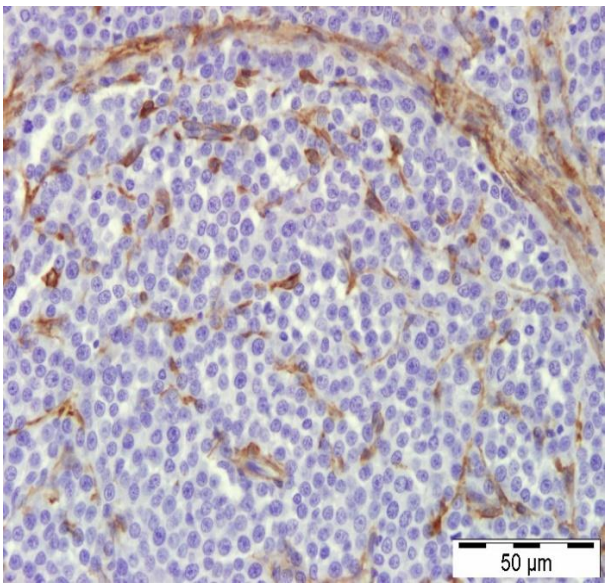

Figure 7. α-Smooth Muscle Actin expression in stromal cells and muscle cells, IHC, Bar=50 µm.

Table 2. IHC marker expressions of all animal

Case No	CD3	CD79αcy	Vimentin	α-Smooth Muscle Actin	S100	Cytokeratin 5/6
Case 1	-	-	+	-	-	-
Case 2	-	-	+	-	-	-
Case 3	-	-	+	-	-	-
Case 4	-	-	+	-	-	-
Case 5	-	-	+	-	-	-
Case 6	-	-	+	-	-	-
Case 7	-	-	+	-	-	-
Case 8	-	-	+	-	-	-
Case 9	-	-	+	-	-	-
Case 10	-	-	+	-	-	-

Discussion

Although TVT is more common in all breeds, 2-8 years old, sexually active free-roaming animals, it has been detected more often in female (64.5%) dogs than in male (35.5%) dogs (Pir Yağcı and Kalender, 2008; Oruç et al., 2011; Oguş and Özmen, 2018). Similar to the literature data, six of the ten TVT cases that constituted our study were female and the mean age was between 3 and 5 (Pir Yağcı and Kalender, 2008; Oruç et al., 2011; Oguş and Özmen, 2018). Although there was no dog breed that TVT showed particularly affinity, most of the dogs in our study were Kangal and Kangal hybrid. In female dogs, the localization of the tumor is usually in the posterior aspect of the vagina, where it usually joins the vestibule. The tumor sometimes surrounds the urethral orifice and can come out of the vulva. In male dogs, the localization of the tumor is mostly on the penis. It can sometimes be observed on the prepuce (Çeşme et al., 2015; Akkoc et al., 2017; Farjanikish et al., 2017). Similar to literature data, we found tumors mostly on the vagina and vulva in female dogs, and on the penis and prepuce in male dogs.

Cytology, histopathology, immunohistochemical staining and molecular methods are used for definitive diagnosis of this tumor (Nak et al., 2004; Park et al. 2006). Macroscopically, tumoral masses are nodular, irregular and fragile, and may show ulceration. The tumor may have cauliflower-like shape, and may also be nodular, papillary or multilobular (Vermooten, 1987; Mısırlıoğlu et al., 1999; Özyurtlu et al., 2008). Microscopically, regular-sized dense cells arranged in solid masses and confluent sheets interlaced by a thin fibrous stroma (Akkoc et al., 2017; Farjanikish et al., 2017). Tumor cells have been found to have vesicular

structures, large oval or round nuclei and prominent hyperchromatic nucleoli, with vacuolar cytoplasm (Nak et al., 2004; Özyurtlu et al., 2008). The nucleus / cytoplasm ratio has been reported to increase in favor of the nucleus (Farjanikish et al., 2017). In some areas, the presence of mononuclear cells in the focal areas between tumor cells has been revealed (Gülbahar and Hazıroğlu, 1995; Park et al., 2006). Increased mitotic activity, anisonucleosis, anisocytosis, necrosis and hemorrhage in tumors are also common findings (Oruç et al., 2011; Özenç, 2016; Oguş and Özmen, 2018). Fibrovascular stroma is scanty in the early stage of the tumor and quite abundant in the following stages (Agnew and MacLachlan, 2017). In cytological examinations, round or oval polyhedral cells are mostly seen with thin cytoplasm with the eosinophilic vacuole, round hyperchromatic nucleus, nucleolus and a reasonable number of mitotic figures (Mısırlıoğlu et al., 1999; Pir Yağcı and Kalender, 2008; Çizmeci et al., 2012; Çeşme et al., 2015). In addition, the ratio of nucleus / cytoplasm increased in favor of the nucleus (Park et al., 2006; Özyurtlu et al., 2008; Ayala-Díaz et al., 2019). TVT cases presented in our study were also evaluated in accordance with the results reported in previous studies in terms of macroscopic, histopathological and cytological features.

The phenotype of TVT is controversial, while some researchers focus on a histiocytic origin, but it is also claimed to be an immature leukocyte phenotype and myeloid origin (Alzate et al., 2019). CD3 antibody shows cellular affinity especially to T lymphocytes (Mukaratirwa and Gruys, 2003). Various researchers have reported that tumoral cells are negative for CD3 expression. They have observed CD3 positive reactions especially in infiltrating lymphocytes located in and around the tumoral tissue (Mozos et al., 1996; Marchal et al., 1997; Pérez et al., 1998; Araújo et al., 2012). The CD3 positive reaction increases in the infiltrating regressive stage of TVT and can be used as an important marker in distinguishing TVT from lymphomas (Mozos et al., 1996). In accordance with the literature data, we detected CD3 positive reactions especially in the cytoplasm of lymphocytes, which are abundant in tumoral areas. It was noteworthy that tumoral cells were negative in terms of CD3 reaction. CD79αcy antibody show cellular affinity especially to B lymphocytes (Pérez et al., 1998). As previously reported (Pérez et al., 1998; Araújo et al., 2012), tumoral cells were negative in terms of CD79 αcy expression. Cells that received a positive reaction were particularly B lymphocytes and plasma cells. In parallel with the literature data, we found that CD79αcy expression in intra-tumoural infiltrating lymphocytes and plasma cells very strong. Vimentin is an important marker of mesenchymal cells and is also expressed by TVT cells (Araújo et al., 2012). In previous studies (Sandusky et al., 1987; Mozos et al., 1996; Marchal et al., 1997; Mukaratirwa et al., 2004; Araújo et al., 2012), the researchers have demonstrated that all TVT cases reacted positively by vimentin immunohistochemically. Similar to literature data, we found the reaction of vimentin positive in all cases. S100 protein is an important protein used in the differential diagnosis between TVT and amelanotic melanoma (Mozos et al., 1996). It has been reported by various researchers that TVT is negative in terms of S100 expression (Sandusky et al., 1985; Sandusky et al., 1987; Mozos et al., 1996; Pereira et al., 2000). Similar to the literature data, we detected S100 negative reactions in all TVT cases. As previously reported (Marchal et al., 1997; Mukaratirwa and Gruys, 2003; Mukaratirwa et al., 2004) α-smooth muscle actin was negative for all TVT cases. We did not detect the α-smooth muscle actin reaction of tumor cells. However, the reaction was observed in stromal cells and and muscle cells from blood vessel walls. Various researchers have mentioned cytokeratin expressions were negative in TVT cases (Sandusky et al., 1987; Mozos et al., 1996; Pereira et al., 2000; Mukaratirwa and Gruys, 2003). We could not determine the cytokeratin 5/6 reaction in tumoral cells.

In conclusion, the negative immunoreactivity of CD3, CD79 αcy+, α-smooth muscle actin and cytokeratin 5/6 in tumor cells suggests that the origin of the tumor is not lymphocytic, smooth muscle cells or epithelial. Only the possibility of histiocytic origin remains. However, some researchers have claimed that the tumor is not of histiocytic origin as a result of their immunohistochemical examinations (Mascarenhas et al., 2014). We conclude that S100 and vimentin are usable markers in the differential diagnosis of TVT. In order to get more precise results, we believe that molecular methods should be used and the number of samples should be increased.

Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the publication of this manuscript.

References

Agnew DW, MacLachlan NJ (2017) Tumors of the genital systems. In : Moulton JE (ed) Tumors of domestic animals, 5th edn, Wiley Blackwell, Iowa, pp 356-424

Akkoc A, Nak D, Demirer A, Şimşek G (2017) Immunocharacterization of matrix metalloproteinase-2 and matrix metalloproteinase-9 in canine transmissible venereal tumors. Biotech Histochem 92:100-106. https://doi.org/10.1080/10520295.2016.1259500

Akpınar E (2010) Malign plevral mezoteyomlarda doku mikroaray yöntemlerle immünohistokimyasal incelenmesi. Uzmanlık Tezi, Ankara Üniversitesi, Ankara, Türkiye

Alzate JM, Montoya-Florez LM, Pérez JE, Rocha NS, Pedraza-Ordonez FJ (2019) The role of the multi-drug resistance 1, p53, b cell lymphoma 2, and bcl 2-associated X genes in the biologic behavior and chemotherapeutic resistance of canine transmissible venereal tumors. Vet Clin Pathol 48:730-739 https://doi.org/10.1111/ vcp.12805.

Araújo MR, Preis IS, Lavalle GE, Cassali GD, Ecco R (2012) Histomorphological and immunohistochemical characterization of 172 cutaneous round cell tumours in dogs. Pesq Vet Bras 32:772-780 https://doi.org/10.1590/ S0100-736X2012000800016

Astsaturov IA, Matutes E, Morilla R, Seon BK, Mason DY, Farahat N, Catovsky D (1996) Differential expression of B29 (CD79b) and mb-1 (CD79a) proteins in acute lymphoblastic leukaemia. Leukemia 10:769 - 773.

Ayala-Díaz S, Jiménez-Lima R, Ramírez-Alcántara KM, Lizano M, Castro-Muñoz LJ, Reyes-Hernández DO, Arroyo-Ledezma J, Manzo-Merino J (2019) Presence of Papillomavirus DNA sequences in the canine transmissible venereal tumor (CTVT). PeerJ 7:e7962. https://doi.org/ 10.7717/peerj.7962

Beverley PCL (1981) Anti-CD3 (T Cell). Eur J Immunol 11: 329-334.

Çeşme H, İpek V, Akkoç A, Salcı H (2015) Bir köpekte primer intranazal transmissible venereal tümör (TVT). Uludag Univ J Fac Vet Med 34:85-88. https://doi.org/10.30782/uluvfd.393266

Çizmeci SÜ, Köse AM, Aydın İ, Dinç DA, Maden M, Köse Sİ (2012) Transmissible venereal tümörlü dişi köpeklerde otojen aşı uygulaması. Eurasian J Vet Sci 28:220-223.

David EE, Rosalie E, George FM, Xiaowei X (2003) Benign pigmented lesions and malignant melanoma. In: David EE (ed): Lever's Histopathology of the Skin. Lippincott Williams & Wilkins, Philadelphia, pp 715-804

Dinç DA (2005) Karnivorlarda infertilite In: Alaçam E (ed): Evcil hayvanlarda doğum ve infertilite. Medisan, Ankara, pp 331-336

Farjanikish G (2017) Morphoathological description of an extra genital canine transmissible venereal tumor. J Fac Vet Med Istanbul Univ 43:74-76. https://doi.org/10.16988/iuvfd.266473

Gülbahar MY, Hazıroğlu R (1995) Bir köpekte ekstragenital metastazlı transmissible venereal tümör olgusu. Ankara Üniv Vet Fak Derg 42:441-445.

Marchal T, Chabanne L, Kaplanski C, Rigal D, Magnol JP (1997) Immunophenotype of the canine transmissible venereal tumour. Vet Immunol Immunopathol 57:1-11. https://doi.org/10.1016/s0165-2427(96)05757-1

Mascarenhas MB, Peixoto PV, Ramadinha RR, Yamasaki EM, Costa SZR, Driemeier D, Sonne L, França TN (2014) Immunohistochemical study of genital and extragenital forms of canine transmissible venereal tumor in Brazil. Pesq Vet Bras 34:250-254. https://doi.org/10.1590/S0100-736X2014000300009

Mısırlıoğlu D, Seyrek İntaş K, Kahraman MM, Özyiğit MÖ, Cangül İT, Sevimli A, Büyükçoban M (1999) Canine transmissible venereal tümör (CTVT) olgularında paramunite aktivatörü baypamun n'in profilaksi ve sağaltım üzerine etkilerinin incelenmesi – makroskobik bulgular. Kafkas Üniv Vet Fak Derg 5:1-8.

Milner RJ (1996) Immunophenotypic classification of canine malignant lymphoma on

formalin-fixed paraffin wax-embedded tissue by means of CD3 and CD79a cell markers. OJVR 63:309-313.

Mukaratirwa S, Chimonyo M, Obwolo M, Gruys E, Nederbragt H (2004) Stromal cells and extracellular matrix components in spontaneous canine transmissible venereal tumour at different stages of growth. Histol Histopathol 19:1117-1123. https://doi.org/10.14670/HH-19.1117

Mukaratirwa S, Gruys E (2003) Canine transmissible venereal tumour: cytogenetic origin, immunophenotype, and immunobiology. A review. Vet Q 25:101-111. https://doi.org/10.1080/01652176.2003.9695151

Mozos E, Méndez A, Gómez-Villamandos JC, Martín De Las Mulas J, Pérez J (1996) Immunohistochemical characterization of canine transmissible venereal tumor. Vet Pathol 33:257-263 https://doi.org/10.1177/ 030098589603300301

Nak D, Mısırlıoğlu D, Nak Y, Seyrek İntaş K, Tek HB (2004) Bir köpekte meme metastazlı transmissible venereal tümör olgusu. Vet Bil Derg 20:99-102.

Oguş E, Özmen Ö (2018) Immunohistochemical determination of the matrix metalloproteinase-2 and -7 expression in transmissible venereal tumor in dogs. MAE Vet Fak Derg 3:106-110. https://doi.org/10.24880/ maeuvfd.481960

Oruç E, Sağlam YS, Cengiz M, Polat B (2011) Bir köpekte bulaşıcı venereal tümör meme metastazının ince iğne aspirasyonu ile sitolojik teşhisi ve vincristine sülfat ile tedavisi. Atatürk Üniversitesi Vet Bil Derg 6:63-69.

Özenç E, Baki Acar D, Birdane MK, Bozkurt MF (2016) Bir köpekte prolapsus vajina ile birlikte gözlemlenen transmissible veneral tümör olgusu. Kocatepe Vet J 9:255-258. https://doi.org/10.5578/kvj.27798

Özyurtlu N, Bademkıran S, Ünver Ö, Yıldız F, İçen H (2008) Dişi bir köpekte transmissible venereal tümörün

abdominal ve subkutan inguinal bölgeye metastazı. Dicle Üniv Vet Fak Derg 1:48-51.

Park MS, Kim Y, Kang MS, Oh SY, Cho DY, Shin NS, Kim DY (2006) Disseminated transmissible venereal tumor in a dog. J Vet Diagn Invest 18:130-133. https://doi.org/10.1177/104063870601800123

Pereira JS, Silva AB, Martins AL, Ferreira AM, Brooks DE (2000) Immunohistochemical characterization of intraocular metastasis of a canine transmissible venereal tumor. Vet Ophthalmol 3:43-47. https://doi.org/10.1046/j.1463-5224.2000.00097.x

Pérez J, Day MJ, Mozos E (1998) Immunohistochemical study of the local inflammatory infiltrate in spontaneous canine transmissible venereal tumour at different stages of growth. Vet Immunol Immunopathol 64:133-147. https://doi.org/10.1016/s0165-2427(98)00131-7

Pir Yağcı İ, Kalender H (2008) Bir erkek köpekte transmissible venereal tumor (TVT) olgusunun vincristine sulphate ile sağaltımı. Kafkas Üniv Vet Fak Derg 14:105-108.

Rosai J (1996) Gastrointestinal tract. In: Rosai J (ed), Ackerman's surgical pathology. Elsevier, Philadelphia, pp 589-816

Sandusky GE, Carlton WW, Wightman KA (1987) Diagnostic immunohistochemistry of canine round cell tumors. Vet Pathol 24:495-499. https://doi.org/10.1177/030098588702400604

Sandusky GE Jr, Carlton WW, Wightman KA (1985) Immunohistochemical staining for S100 protein in the diagnosis of canine amelanotic melanoma. Vet Pathol 22:577-581
https://doi.org/10.1177030098588502200611/.

Sudjaidee P, Ponglowhapan S, Tummaruk P, Chatdarong K (2015) Factors affecting treatment outcome of canine transmissible venereal tumor: a retrospective study. Thai J Vet Med 45:535-541.

Tosun İ (2009) Gastrointestinal stromal tümörlerde immünohistokimyasal yöntemle saptanan P16 protein ekspresyonunun prognostik önemi. Uzmanlık Tezi, Haydar Paşa Numune Eğitim Araştırma Hastahanesi, İstanbul, Türkiye

Uçar M (2016) Transmissible venereal tumor: a review. Kocatepe Vet J 9:230-235. https://doi.org/10.5578/kvj.26524

Vermooten MI (1987) Canine transmissible venereal tumor (TVT): a review. J S Afr Vet Assoc 58:147-150.

Yuan SM (2015) α-Smooth Muscle Actin and ACTA2 Gene Expressions in Vasculopathies. Braz J Cardiovasc Surg 30:644-649. https://doi.org/10.5935/1678-9741.20150081