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Case Report

Ocular Transmissible Venereal Tumor in Two Dogs: Clinical and Cyto-histopathological Evaluation

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Abstract

Transmissible venereal tumor (TVT) is a transmissible cancer that typically affects the external genital organs in canines. It can spread to other parts of the body via auto- and hetero-transplantation in a way that neoplastic cells are inoculated into the degraded mucosa or skin during the interaction of the animals, such as licking and sniffing, or less commonly via hematogenous or lymphatic routes. Here, we presented the clinical, cytological, and histopathological characteristics of two rare cases of TVT that developed ocular involvement and were associated with a poor prognosis. Fine needle aspiration biopsy was the initial method of choice in the diagnosis which is rapid, minimally invasive, and effective. It was diagnostic in one case and aided in the diagnosis in the other. The latter was considered a malignant round cell tumor in the cytological examination that needed further clinical and histopathological examination for definitive diagnosis. TVT is a round cell tumor that should be considered in the differential diagnosis of ocular lesions in canines.

Keywords: *Transmissible venereal tumor, TVT, Eye, Dog*

İki Köpekte Oküler Bulaşıcı Veneral Tümör: Klinik ve Sito-histopatolojik Değerlendirme

Öz

Bulaşıcı veneral tümör (BVT), köpeklerde tipik olarak dış genital organları etkileyen bulaşıcı bir kanser türüdür. Vücudun diğer kısımlarına oto- ve heterotransplantasyon ile yayılabilir. Bu yayılım hayvanların etkileşimi sırasında, yalama ve koklama gibi, hasarlı mukozaya veya deriye ekilen neoplastik hücreler ile ya da daha az rastlanan hematojen veya lenfatik yollarla olabilir. Bu yazımızda, oküler tutulum geliştiren ve kötü prognoz gösteren iki nadir BVT olgusunun klinik, sitolojik ve histopatolojik özelliklerini sunduk. İnce iğne aspirasyon biyopsisi hızlı, minimal invaziv ve efektif olmasıyla tanıda ilk tercih edilen yöntemdi. Bu yöntem, bir olgumuzda tanısal iken, diğer olguda tanıya yardımcı oldu. İkinci olgunun sitolojik incelemesinde malign yuvarlak hücreli tümör düşünüldü ve daha ileri klinik değerlendirme ve histopatolojik inceleme ile kesin tanıya gidildi. BVT, köpeklerde oküler lezyonların ayırıcı tanısında akılda tutulması gereken yuvarlak hücreli bir tümördür.

Anahtar sözcükler: *Bulaşıcı veneral tümör, TVT, Göz, Köpek*

INTRODUCTION

Transmissible venereal tumor (TVT) is a neoplasm that particularly involves the external genital organs in dogs. It is common among free-ranging dogs that suffer from malnutrition and live in groups in tropical and subtropical regions. The disease is transmitted to young and sexually mature dogs through coitus [1]. While spontaneous regression can be observed in some dogs infected with TVT, others may have a clinical picture that can lead to metastasis and even death [2]. When TVT cases are left untreated for a long time, metastases have been reported to occur usually to the lips and buccal and nasal mucosa; whereas metastases to the kidney, spleen, mesenteric lymph nodes, liver, pancreas, tonsils, lungs, brain, mediastinum, pituitary gland, skin, regional lymph nodes, and eyes were less

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common [3-7]. We aimed to explain the clinical, cytological, and histopathological characteristics of TVT in two dogs located in the anogenital region, in addition to bilateral intraocular and palpebral conjunctiva involvement in one dog and multiple organ metastases including nictitating membrane and bilateral palpebral involvement in the other.

CASE HISTORY

Case 1: A 4-year-old non-castrated female pointer mix was admitted to the Near East University Animal Hospital with loss of vision and bilateral eye redness. Clinical examination showed bilateral chemosis, photophobia, epiphora, blepharospasm, and 180-degree corneal vascularization originating at the limbus. There were buphthalmos, corneal edema, and episcleral congestion due to bilaterally increased intraocular pressure. Physical examination revealed negative direct-indirect pupillary light reflexes and positive corneal reflex. In the direct ophthalmoscopic examination of the anterior chamber, neoplastic uveal masses were detected at 11-03 o'clock iridal quadrant in the right eye and at 08-12 o'clock iridal quadrant in the left eye in addition to a conjunctival mass in the left upper palpebra *(Fig. 1-a,b)*.

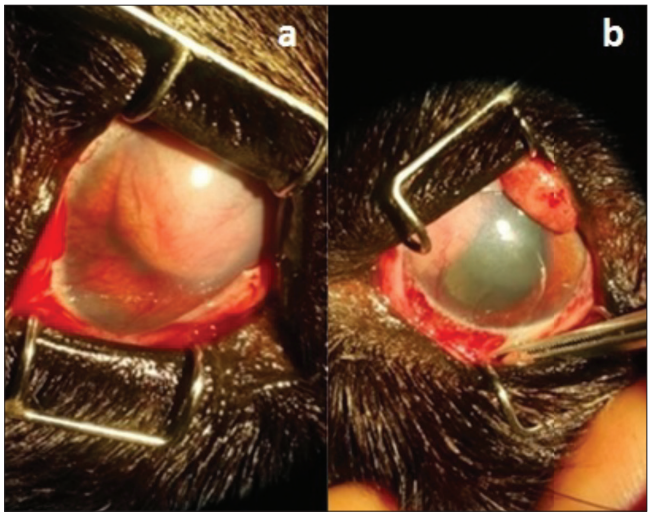

Fig 1. Appearance of uveal TVT in the right eye **(a)**; appearance of uveal, upper eyelid, and conjunctival TVT in the left eye of case 1 **(b)**

Abdominal and thoracic imaging results were normal; however, masses were detected in vaginal and rectal examinations. Ocular ultrasonography (USG) examination was performed with a transpalpebral approach using a 9 MHz microconvex probe under sedation, and sagittal, dorsal, and transversal sections were obtained. Ocular USG revealed a solid mass at the ciliary body level and retinal detachment in both eyes *(Fig. 2, Fig. 3)*. A complete blood count, biochemistry, and serology analyses were within the normal range.

Fine needle aspiration biopsy (FNAB) was performed in the anterior chamber and vitreous body by aspirating 0.1 mL material for cytological examination [8] *(Fig. 4)*. Touch and smear preparations were made from the aspirates, fixed in 96% ethanol and stained with Papanicolaou stain. Microscopically, the slides were sufficiently cellular and composed of monotonous neoplastic cells forming small, poorly cohesive groups or dispersed in an isolated fashion. The cells had round to oval nuclei and single prominent nucleoli. Although most of the tumor cells had large cytoplasm, occasional cells had an increased nuclear-cytoplasmic ratio. In some cells, intracytoplasmic brown pigment compatible with melanin was encountered. Several mitotic figures were identified. Cytomorphologic findings were consistent with a group of tumors known as "malignant round cell tumors," and melanoma was suspected primarily due to the tumor location and the presence of pigmented cells. At the time of cytological evaluation, a physical examination revealed two other masses in the anal and vaginal areas. With this clinical finding, TVT, another blue round cell tumor, was also considered at the top of the differential diagnosis list. The

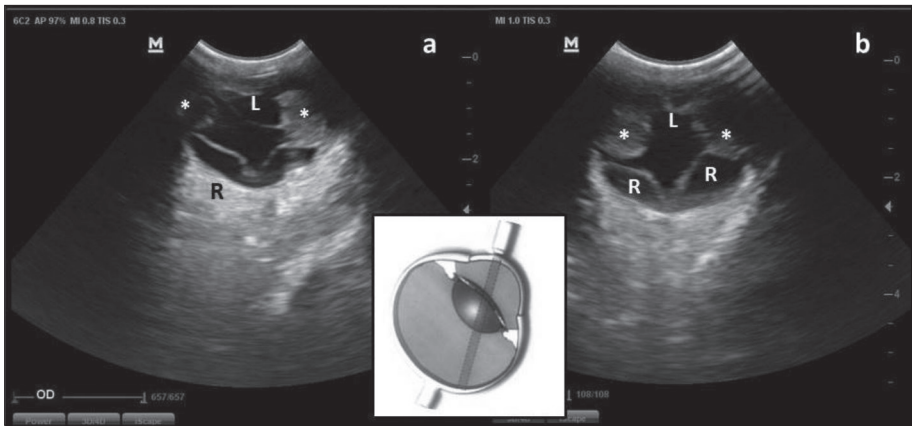

Fig 2. Homogenous and moderately echoic, solid masses (*) at the level of the ciliary body taken from right eye of case 1 and «flying seagull» appearance of the retinal (R) detachment **(a)**; Advanced retinal detachment (R) and echogenic masses (*) protruding from the caudal aspect of the ciliary body to the vitreous **(b)**; L: Lens

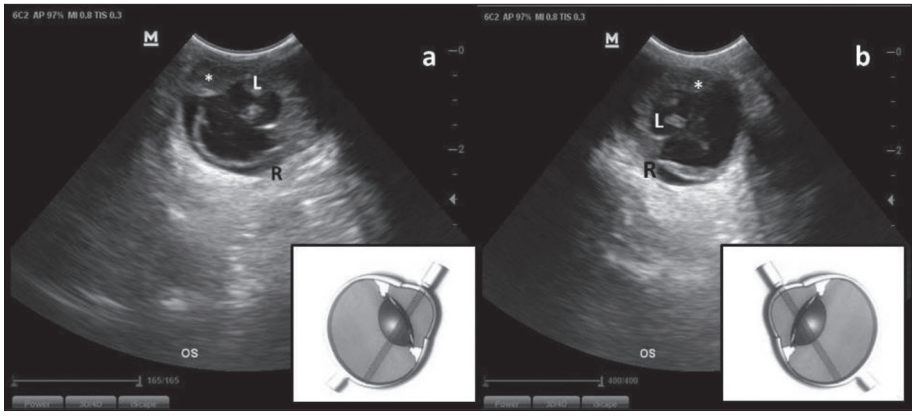

Fig 3. In the nearly sagittal sections of the left eye of case 1, a homogeneous, moderately echoic, solid structure (*) protruding towards the anterior chamber at the level of ciliary body was observed. The echogenic structures in the lens (L) were noted as two separate clusters. The retinal layer (R) appeared as a hyperechoic line that was thickened and separated from the ground **(a)**; The appearance of the left eye of case 1 from a different aspect, with an echogenic mass spreading into the anterior chamber (*). The mass may be originating from the iris or ciliary body. Hyperechoic structures within the lens (L) may indicate a cataract onset or swelling of the lens. Foci of irregular echogenic reflex and membranes are also seen in the corpus vitreum, which is expected to be anechoic. These are thought to be caused by degeneration or posterior separation of the vitreous. In addition, retinal detachment (R) and/or retinal retraction results in chorioretinal thickening **(b)**

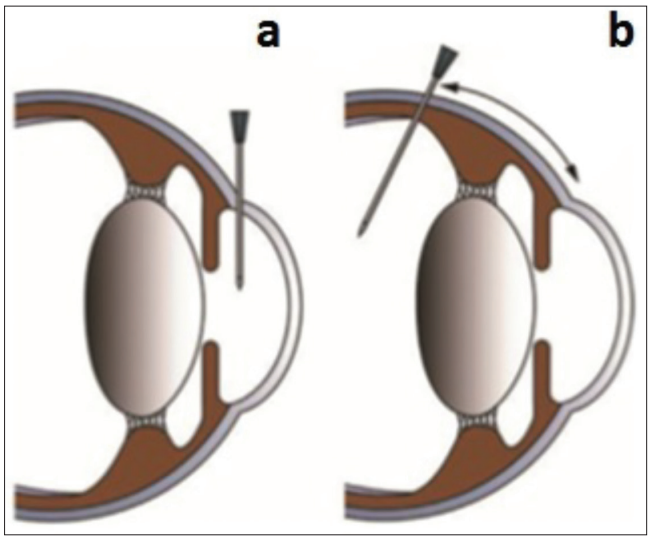

Fig 4. FNAB entrance points at the anterior chamber **(a)** and vitreus **(b)**

patient was considered to have malignant cytological findings consistent with a malignant round cell tumor *(Fig. 5-a)*.

Bilateral bulbus oculi extirpation was performed due to the rapid growth of intraocular masses and retinal detachment. Both eyes and two other anogenital masses were excised and immediately fixed in 10% neutral buffered formaldehyde solution. Grossly, the cut surface of the tumors was gray-white, solid, and nodular. In the left eye, the tumor was located predominantly in the anterior chamber, whereas the right eye was entirely involved and penetrated by the tumor *(Fig. 5-b)*. The sampled tissues were processed using a fully automated tissue processing machine, then embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin. Histological examination revealed solid or trabecular sheets of large and monotonous round cells with a single prominent nucleolus. The cells had a moderate amount of eosinophilic to clear cytoplasm. Some cells contained clear cytoplasmic vacuoles. There were an arborizing fibrovascular network and scant stroma among the tumor cells. Melanin pigment was found to be dispersed from the iris *(Fig. 5-c)*. The anal and vaginal tumors looked blander with very few mitoses and without necrosis, while the tumors located in the eyes showed more pronounced mitotic activity and foci of necrosis in the areas corresponding to fibrovascular septa *(Fig. 5-d)*. Clinical, cytological, and histopathological findings were consistent with a diagnosis of TVT. Vincristine was recommended; however, the patient died within 1 month after refusing treatment.

Case 2: A 5-year-old male dog was admitted to the Near East University Animal Hospital with a tumor located in bilateral palpebra and subcutaneous spread of the tumor in the whole body. Examination of the eyes revealed no intraocular problems; however, there was bilateral conjunctival hyperemia, chemosis, masses with varying sizes on the edges of lower and upper eyelids, hyperplasia and a subsequent prolapse in the left nictitating membrane *(Fig. 6-a, Fig. 7-a)*. As no intraocular lesion was detected in this case, FNAB was not performed. In the clinical examination, subcutaneous masses with varying sizes were detected in the head, neck, abdomen, extremities, and penis. Abdominal and thoracic radiographs and USG examination showed involvement of the liver and spleen as well as multilobular heterogenous masses in the vicinity of paraaortic, iliac, and inguinal lymph nodes. There was a prominent thickening of the submucosal layer

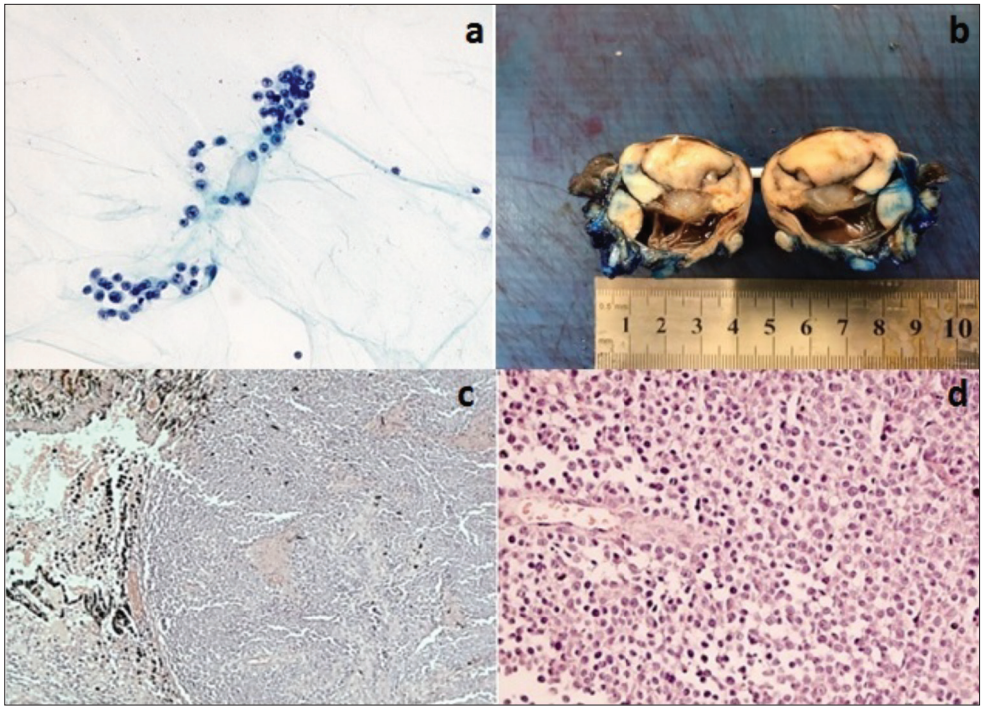

Fig 5. Cytologically, isolated or small groups of tumor cells with round nuclei, prominent nucleoli, and moderate cytoplasm are present **(a)**; Gray-white nodular tumor with a solid appearance. Extensive intraocular involvement is seen **(b)**; Histopathological examination of the excised eyes showed a round cell tumor in a sheet-like pattern. There are fibrovascular cores among tumor cells with foci of necrosis. Melanin pigment which was confusing in the cytologic examination found to be originating from the melanocytes in the iris **(c)**; Monotonous round tumor cells and rare mitoses in the histopathological examination of the masses excised from the anal and vaginal regions **(d)**

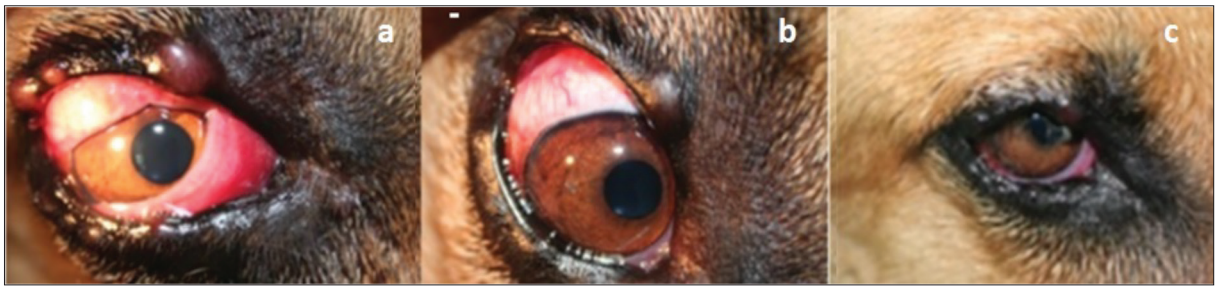

Fig 6. Significant tumor masses of TVT on the eyelid margin in the right eye of case 2 **(a)**; Shrinkage of the masses following the first **(b)** and second vincristin application **(c)**

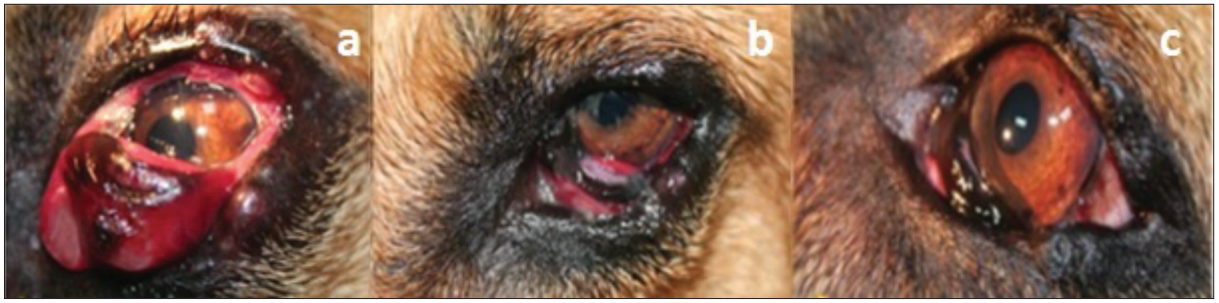

Fig 7. In the left eye of case 2, TVT-induced third eyelid hyperplasia and associated prolapse **(a)**; Shrinkage of the masses following the first **(b)** and second vincristin application **(c)**

of the stomach and small intestine. Ocular USG revealed irregularity and thickening in both bulbus oculi at the lateral ciliary body level. Fine needle aspiration biopsy was performed on one of the skin lesions, and slides fixed in 96% alcohol were stained with PAP, whereas air-dried slides were stained with Giemsa. Cytological evaluation revealed mostly isolated or sporadic groups of neoplastic cells with round nuclei and prominent nucleoli that exhibited slight pleomorphism. The cells had a moderate amount of eosinophilic cytoplasm and contained multiple small and clear vacuoles. There were occasional mitotic figures. Cytomorphologic findings were consistent with TVT. Vincristine (Vincristine 2 mg vial, Koçak Farma) 0.075 mg/m^2 was administered intravenously once a week for a total of 10 times and doxorubicin hydrochloride (Adriamycin 50 mg vial, Saba İlaç San.) 25 mg/m^2 iv was administered every

3 weeks for a total of three times. The masses were found to gradually shrink after the first and second administration *(Fig. 6-b,c, Fig. 7-b,c)*. Approximately three months after the start of treatment, the patient died following bulbus oculi extirpation due to intraocular metastasis of TVT, despite the treatment administered to treat severe uveitis that developed in both eyes and continued vincristine administration.

DISCUSSION

Although it has been reported that TVT primarily involves the genital region, it could also involve other parts of the body without genital findings when animals lick and sniff the genital areas of infected animals [5,7,9]. Metastases are caused by spread from a genital tumor primarily via autotransplantation or heterotransplantation due to neoplastic cells inoculated into the degraded mucosa or skin through licking, sniffing, itching, and biting, or less commonly via hematogenous or lymphatic routes [10,11]. Some researchers highlighted that TVT should also be included in the differential diagnosis if there are extragenital, especially ocular masses in dogs living in geographical regions with a high TVT prevalence to avoid such incidents [12,13]. Metastatic ocular TVTs involving the cornea and sclera and primarily ocular TVTs localized in the nictitating membrane and bulbar conjunctival tissue have been reported [8,10,14,15]. Although to a lesser extent, metastatic lesions resulting in widespread intraocular damage have also been reported [10,16,17]. TVT could be confused with various eye diseases such as severe uveitis and glaucoma when TVT is solely present in extragenital regions like the eyes in the absence of a simultaneous genital tumor [14,15,18]. In cases with intraocular neoplasms, USG examination is important for showing the involvement of the ciliary body and posterior structures by anterior uveal tumors. Bulbus oculi resection performed before the tumor spreads to the orbit in blind and painful eyes has been reported to be therapeutic [19,20]. In both cases, lesions were not noticed until palpebral and intraocular metastases occurred, which led to delayed admission and, in turn, delayed diagnosis and treatment. Intraocular USG; benefited in the decision of the extirpation of blind and painful eyes. It has been reported to cause tumor regression with TVT cell lysates by inducing canine dentritic cells [21,22]. In cases with conjunctival and palpebral TVT it has been stated that surgical excision and vincristine application may be curative [14]. Intratumoral therapy of vincristine and IL2 has also been reported to have impressive therapeutic effects on TVT [23,24]. Although chemotherapy is thought to be effective in preventing spread, it is believed that it does not benefit tumors with intraocular localization. In recent studies additionally to histological and cytological analyzes, LINE-c-myc PCR test is envisaged for the diagnosis of extragenital TVT [25]. The diagnosis of TVT relies on the clinical, cytological, and/or histopathological findings [1]. Grossly, TVTs in the genital area have a mean diameter of 4.0-7.5 cm, they bleed easily; have an irregular, cauliflower-like, pedunculated shape; and are friable. Extragenital lesions have an irregular nodular or multilobular appearance with a solid cut surface and whitish color [2]. Histopathologically, TVT is composed of monotonous tumor cells, which are poor in stroma and have sheet-like or trabecular alignment in the arborizing fibrovascular network. The tumor cells are sometimes accompanied by lymphocytes, plasma cells, and macrophages [1,6,14]. Cytomorphologically, TVT cells are typically uniform, large and round or slightly polyhedral. They have round to oval, centrally located nuclei, high nuclear-cytoplasmic ratio, and generally have one prominent basophilic nucleolus. The cytoplasm is generally large and typically has a varying number of clear vacuoles. Fine needle aspiration biopsy should be the primary choice in suspected TVT cases, as it is a minimally invasive, inexpensive, and fast technique that provides diagnostic information [11]. In cases where a definitive diagnosis cannot be made with cytology, diagnosis can be made through examining the tissue biopsy specimen with histopathologic and immunohistochemical stains. TVT must be distinguished from other round cell tumors, i.e., histiocytoma, lymphoma, mast cell tumors, poorly differentiated carcinoma, and melanoma [10,11,14]. In a study, an algorithm was used for the differential diagnosis of TVT. It was reported that the presence of cytoplasmic granules in the presence of discrete round cells indicated a mast cell tumor, whereas the absence of cytoplasmic granules along with the presence of vacuolization indicated TVT. In the absence of cytoplasmic granules in neoplastic cells, the presence of bean-shaped nuclei with indented appearance indicates histiocytoma, whereas the presence of giant cells indicates histiocytic sarcoma. Since there was no pronounced ocular mass in Case 2, FNAB samples were taken from one of the skin lesions and the typical cytomorphological findings were diagnostic. Whereas, in Case 1, a diagnosis of malignant round cell tumor could be made based on the cytological features of the tumor obtained via aqueocentesis and hyalocentesis. In this case, a straightforward diagnosis of TVT could not be made for three reasons: i) atypical location of the tumor, ii) lack of evident cytoplasmic vacuolization in tumor cells, which was previously reported as a diagnostic difficulty in cytology samples, and iii) the detection of pigments consistent with melanin in some cells led to a melanoma suspicion [10]. A subsequent physical examination and imaging studies revealed tumors in the anogenital region. In the histopathological examination of both intraocular and anogenital tumors, melanin pigment was found to be dispersed from the iris instead of being produced by tumor cells, and the tumor demonstrated typical features of TVT consisting of monotonous tumor cells with some of them having vacuolated large eosinophilic or clear cytoplasm, single prominent nucleoli in a background of thin fibrovascular network. The diagnosis of TVT was made based on the combination of clinical, cytological, and histopathological findings. In dogs, TVT can spread

to other parts of the body aside from the genital organs via auto- and hetero-transplantation. In patients with ocular involvement, the owners generally present to a clinic due to the presence of an externally visible tumor in the bulbus oculi and surrounding tissues or loss of vision. In the presence of intraocular masses larger than 2 mm, cytological examination of FNAB samples collected from the anterior and vitreous chambers has been reported to be practical and beneficial in the diagnosis. On the other hand, histopathological examination is recommended in cases when it is not possible to sample by FNAB or make a definitive diagnosis on cytological examination. In the presence of intraocular tumors, USG is useful in determining whether the eye is functional and deciding whether to perform a bulbus oculi extirpation. It was observed that chemotherapy provided a temporary improvement but failed to provide a cure in cases with metastases to the eye and the surrounding tissues.

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