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Cerebral metastasis of transmissible venereal tumor after effective chemotherapy in a dog

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Abstract: A male mixed breed street dog (age unknown) presented with an ocular mass and multiple ulcerated subcutaneous nodules on both hind limbs which bled easily when touched. Inspection of the genital organs revealed that no masses were present. The ocular mass and nodules were diagnosed as a transmissible venereal tumor (TVT) by fine-needle aspiration cytology. The total remission of the tumor was achieved after 5 applications of vincristine sulfate. The dog was adopted during the remission period, but 5 months after adoption he developed neurological signs and died. The necropsy revealed a mass located in the trunk of the corpus callosum of the right hemisphere of the brain. The cytological, histological, and PCR findings of the brain mass were consistent with a TVT. These findings highlight the need to consider the aggressive metastatic potential of canine TVT, even after apparently effective chemotherapy.

Key words: Cerebral, dog, metastases, TVT, vincristine

1. Introduction

Transmissible venereal tumor (TVT) is a neoplasm of round cells that occurs in dogs. A recent study of the University of Cambridge found that the distribution is correlated mostly with the presence of street dogs that mate freely [1]. The tumor is transmitted via direct transplantation of viable cells during sexual intercourse. TVT prevalence is greater in populations of young, sexually active dogs [2,3]. A study of TVT prevalence in dogs in Mexico City found that the age at presentation was typically 3–4 years old [4]. Dogs with a suppressed immune system are more likely to have a more severe disease [2,5,6]. Due to the mode of transmission, this neoplasm mainly affects the genitals [5–7], although parenchymal organs and even the brain can be affected [8,9]. There are few reports of this neoplasm affecting extragenital locations. Most reports describe tumors in anatomical locations that can be associated with social behaviors, including sniffing, scratching, and biting [6,7,10]. Among the few rare reported cases of extragenital primary TVT [6,10,11], nasal, oral, and subcutaneous TVTs without evidence of genital tumors have been found [3,6,7,11]. The values for incidence of metastasis are between 1% and 5%. Metastatic TVTs have been found in peritoneal fat, tonsils, eyes, liver, spleen, lungs, kidneys, regional lymph nodes, skin, muscle, and brain tissues [1,2,5,7]. When present in brain tissues, ataxia and other neurological signs (e.g., loss of vision, smell, and hearing) occur, even after an effective treatment. TVT is typically diagnosed using histological or cytological studies, or both. The treatment for this neoplasm is based on the application of vincristine sulfate at weekly intervals for a total of 4–8 applications; this protocol generally results in tumor remission [1,3,6,7,11].

2. Case history

A mixed breed, young male street dog of unknown age was brought to the Veterinary Pathology Laboratory at the Autonomous University of Sinaloa. He was undernourished and had severe purulent conjunctivitis and an ocular mass in the right eye (Figure 1A). Abundant subcutaneous ulcerated nodules of 1–2 cm in diameter that bled easily when touched were found on both hind limbs (Figure 1B). Due to the presence of the ocular mass, the nodule's characteristics, and the dog's history, a TVT was suspected. However, an inspection of the genital area revealed no masses. Fine-needle aspiration cytology was performed. A microscopic examination revealed abundant round cells showing a moderated cytoplasmic-nucleus relationship, prominent nucleoli, and, in some cells, cytoplasmic vacuolization. Cells with atypical mitosis were abundant. These findings were consistent with TVT. A chemotherapy protocol with vincristine sulfate at a dose of 0.7 mg/m^2

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was initiated. The drug was given intravenously at weekly intervals. There was a noticeable improvement by the third application, including a significant decrease in the size of the ocular mass. Five applications were necessary for total remission of the tumors (Figure 1C). Once recovered, the dog was adopted. Five months after adoption, the dog was observed walking in circles. He was hospitalized with ataxia and anorexia, where progressive loss of sight, hearing, and smell was also observed, and he died 15 days later. A necropsy revealed a 2.5 cm diameter nodular lesion in the trunk of the corpus callosum of the right hemisphere of the brain (Figure 2A). The cytological findings were compatible with TVT (Figure 2B). Histologically, solid tissue was found that had rich conjunctival stroma in some areas. The round cells were abundant in cytoplasm and were vacuolated with prominent nucleoli and perinuclear chromatin. The cells were arranged in solid groups and in some areas they formed beads. In addition to discrete pockets of lymphoid cells, abundant mitoses and some apoptotic cells were present (Figure 2C). The histological diagnosis was TVT. Polymerase chain reaction (PCR) was used to confirm the presence of TVT. A commercial

Figure 1. A) Cauliflower-like mass in the right eye. B) Multiple subcutaneous nodules in hind limbs with characteristics that are consistent with a transmissible venereal tumor. An overall poor condition of health was apparent. C) Total remission of the ocular mass and of the nodules on the hind limbs occurred after vincristine sulfate chemotherapy. There was also a clear improvement in the overall condition of the patient, including body weight, skin, and coat.

extraction matrix (QIAamp) was used to extract DNA from the tissue found in the brain. Primer sequences specific to the regions of the LINE-c-*myc* gene rearrangement MycS-2 (5′-ATTCCTACGAATGAATGATTGGCCAGA-3′) and LINE AS-1 (5′-CAGACACATAGATCAGTGGAACAGAAT-3′) were used [12]. Amplification was performed using a thermocycler (Bio-Rad, MJ Mini Personal Thermal Cycler). The mixture for amplification contained 5 µL of DNA mold, 1 µL (10 pmol L^{-1}) of each of the oligonucleotides (forward and reverse), 12.5 µL of PCR SuperMix (22 mM Tris-HCl, 55 mM KCl, 1.64 mM $MgCl_2$, 220 M dGTP, 220 M dATP, 220 M dTTP, 220 M dCTP, 22 U/mL Taq DNA recombinant polymerase), and 1.5 µL of $MgCl_2$ (50 mM). Nuclease-free water was added to obtain the final volume of 25 µL. The PCR thermal cycling consisted of 35 cycles of 94 °C for 1 min (denaturation), 64 °C for 50 s (annealing), and 72 °C for 1 min (extension). The PCR products (desired amplicon was 550 bp) were then resolved using 1.5% (w/v) agarose gel electrophoresis in 1.5% agarose gels predyed with GelRed (solution 1:10,000) and in electrophoresis buffer of 0.5X Tris-EDTA. A molecular weight marker of 100 bp (Bio-Rad) was used, and the gels were examined in an imaging system (Gel Documentation System EZ GelDoc) and photographed for analysis (Figure 2D).

3. Results and discussion

The cytological, histological, and PCR findings of the brain mass were consistent with a TVT. Dogs with suppressed immune systems have increased severity of disease

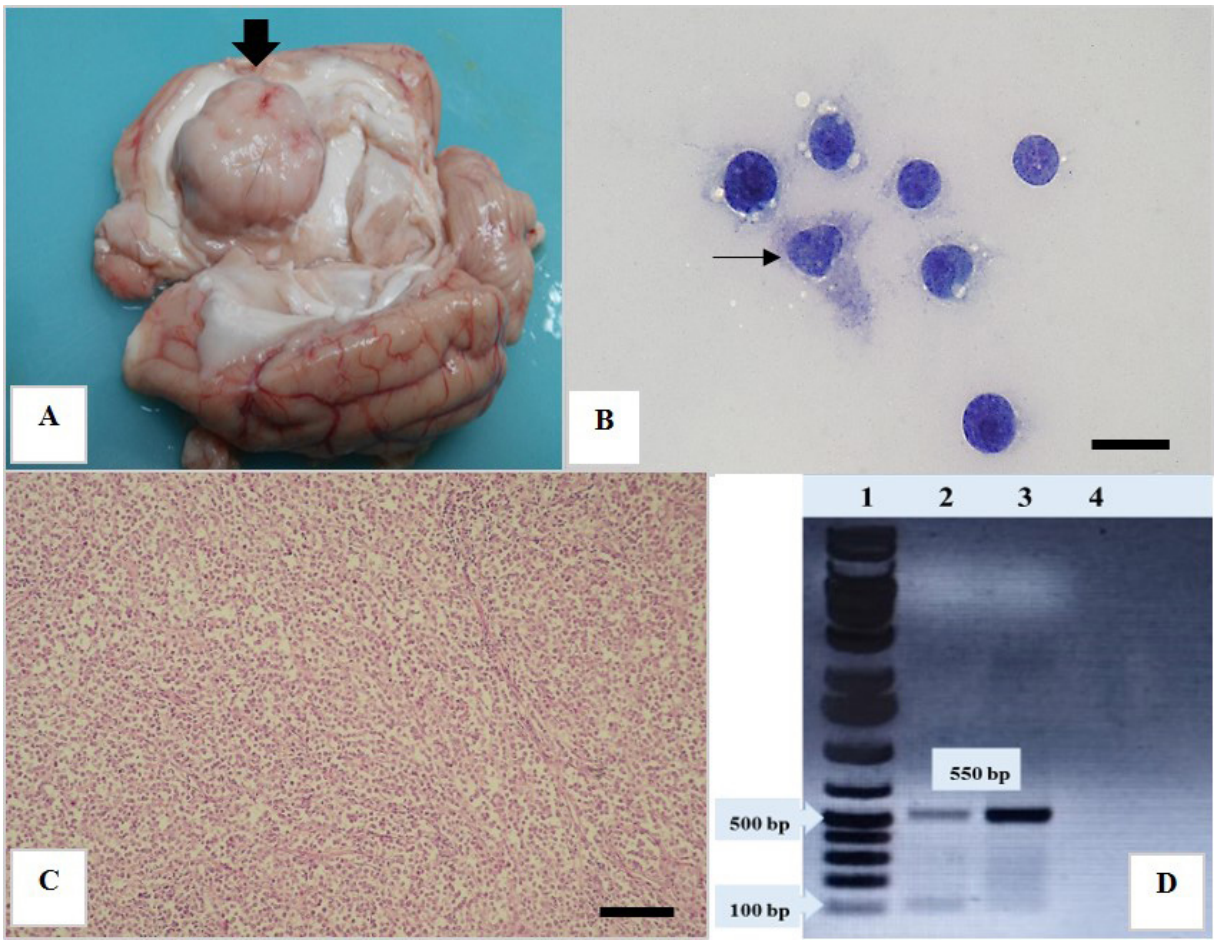

Figure 2. A) A brain mass of 2.5 cm diameter in the right hemisphere, which was sampled using fine-needle aspiration (black arrow). Histopathology and cytology were used to determine the characteristics of the mass. B) On cytology, the TVT cells showed a predominance of round cells with abundant pale cytoplasm and distinct, punctate cytoplasmic vacuoles. Large neuron cell body (thin arrow). Papanicolaou stain. Bar: 20 µm. C) Histopathology image with round cells with abundant cytoplasm, vacuolated, with prominent nucleoli and perinuclear chromatin, divided into packets by fine fibrous stroma. H&E. Bar: 50 µm. D) The PCR results for detection of the region associated with the LINE-c-*myc* gene rearrangement MycS-2 and LINE AS-1. Lane 1, MPM, 100 bp; lane 2, positive control; lane 3, DNA from nodular lesion found in the trunk of the corpus callosum; lane 4, negative control.

[2,5,6,13], which is compatible with the characteristics of this dog exposed to severe malnutrition that likely caused immunosuppression. In some cases, spontaneous regression of the tumor has been observed in healthy animals with vigorous immune systems [14]. The genitals of this dog were without a neoplastic TVT lesion, which suggests that the origin of the primary neoplasm was the skin. The presence of the eye mass and the nodules on the hind legs may be associated with social behavior during mating [6,7,10]. Before being adopted the dog was living on the streets and would have been exposed to a high risk of TVT transmission. With respect to the cellular spread of the tumor, there are other reports of TVT cerebral metastasis, but in all of them, the primary neoplasm was located in the genitals [8,9]. Reports of extragenital primary TVT, however, are rare [6,10,11]. Among the few cases of this type, nasal, oral, and subcutaneous TVTs without evidence of genital tumors have been observed [3,6,7,11]. Reports of metastasis are less than 1%–5%. When metastasis does occur, it has been found in peritoneal fat, tonsils, eyes, liver, spleen, lungs, kidneys, regional lymph nodes, skin, muscle, and brain tissues [1,2,5,13]. In the present case, ataxia and other neurological signs such as loss of vision, smell, and hearing were likely associated with the neoplastic lesion in the trunk of the corpus callosum of the right hemisphere of the brain. The neurological signs continued even after successful treatment with vincristine sulfate. Pinczowski et al. reported similar results after vincristine sulfate treatment of a dog that also presented with neurological disturbances [8]. The location of the tumor that affected this dog, however, corresponded to what has been found in other cases; exposure of the area with tumor cells can occur because large numbers of blood vessels are present in this area of the brain [8]. Treatment is based on the application of vincristine sulfate to doses of 0.025 mg/kg IV or 0.5–0.7 mg/m^2 at weekly intervals for 4 to 8 applications; use of this protocol generally results in tumor remission [1,3,6,7,13]. In this case, total remission was achieved after 5 doses, without the need for complementary treatments. There was no resistance associated with the treatment. Despite the successful result of the initial therapy using vincristine, viable tumor cells likely spread from the eye to the brain tissues due to the close anatomical proximity and then caused the nervous system alterations and the subsequent death of the patient. This report presents a case of canine TVT with metastasis to brain, even after effective chemotherapy and without having any genital tumor. In cases of this type of presentation, clinicians should consider the other round cell tumors as differential diagnoses (histiocytoma, histiocytic sarcoma, mast cell tumor, plasma cell tumor, and cutaneous lymphoma), even when there is no macroscopic involvement of the genital organs. Because TVT neoplastic cells can spread to the central nervous system, in patients with neoplastic lesions in the conjunctival mucosa, periodic follow-ups and clinical reviews should be performed after apparent complete remission of neoplasia with chemotherapy.

Conflict of interest

The authors declare that there is no conflict of interest associated with the publication of this manuscript.

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