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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; SP-9(9): 126-130

www.thepharmajournal.com
Received: 20-07-2020
Accepted: 26-08-2020

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Sticker tumour (Transmissible venereal tumour) in dog

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DOI: https://doi.org/10.22271/tpi.2020.v9.i9Sc.5188

Abstract
Sticker tumor or Transmissible Venereal Tumor (TVT) is one of the important neoplasms of dogs. It is mainly prevalent in all dog breeds. TVT affects dogs of 2-5 years age group predominantly and both sexes get equally affected. Compromised immune system plays main role in acquiring and spreading of TVT. External genitalia are the prime site for this tumor; but occasional internal metastasis is also reported. It transmits mainly through coitus. Fine needle aspiration (FNA) cytology is the easiest and quite reliable way of diagnosing TVT cells. Wet fixation cytology smears and histopathology (H&E) are also useful methods. Since TVT cells resemble histiocytic types and considering cell lineage differences; proper differentiation of the tumor can be challenging. Molecular markers and other features like mitotic index and immune cells infiltration are used for proper differentiation; aggressiveness and immune response to the TVT. Recent DNA fragment technique helped to classify TVT into plasmacytic; lymphocytic and mix types. Vincristine chemotherapy is main treatment option for TVT along with surgical; radiological and immunotherapy methods.

Keywords: Sticker cell, TVT, FNA cytology, plasmacytic, DNA fragmentation, vincristine, introduction

Introduction
The dog is a vital household pet. Sticker tumour or transmissible veneral tumor (TVT) is widespread among 2-5 years of age (Higgins, 1966) varying from 23-43% of the total tumors in dogs, TVT is the most numerous tumor in India.
TVT being the most common canine tumor (Das and Das, 2000) [10], is prevalent in all dog breeds of tropical and subtropical climate regions (Goldschmidt, 2002) [15]. Foxes, coyotes, and jackals, which are members of the Canine family, are also susceptible (Gruys, 2003) [30].
TVT is apparent equally in both male and female dogs, posing severe issues and concerns around the world. As immunologically compromised animals are prone to more severe conditions. The host immunologic response competence plays a crucial role in the expansion of such tumors. Transmissible venereal tumor (TVT) is popularly known as sticker tumor along with other names such as venereal granuloma, canine condyloma, transmissible lymphosarcoma, or infectious sarcoma (Goldschmidt, 2002) [15].
It is naturally occurring and horizontally transmitted highly contagious cancer (Murgia *et al*.., 2006) [31] transmitted during coitus, and it is explained as a benign reticuloendothelial tumor or round cell benign neoplasia of the dog (Goldschmidt, 2002) [15]. Primarily, small tumor lesion consequently progresses to a large, ulcerated, and contaminated mass (Das and Das, 2000) [10] with hemorrhagic discharge followed by offensive odour (Do Amaral *et al.*, 2007) [12] limiting to the external genitalia mucous membranes of both sexes of any breeds. Occasionally, TVT localization can be seen in the uterus. In dog, the chromosome number is 78, with two acrocentric chromosomes. In TVT, there are morphology and numerical aberrations of the constituent cell's chromosomes with 58-59 chromosomes and 13-17 metacentric along with 42 acrocentric chromosomes. While growing larger, tumors bleed and become contaminated quickly. Young and sexually mature animals are more prone to TVT, as they are being relocated during coitus with intact viable cells passing through major histocompatibility complex (MHC) barriers within the same species (Mukaratirwa and Gruys, 2003) [30].
Between the stages of tumor progression, differences in cell types have been found (Higgins, 1966). In progressive tumors, growth has round cells with microvilli, while regressing tumors has transitional fusiform cells. TVTs can grow unpredictably slowly or become invasively malignant and metastasize (Moulton, 1978) [29]. Mucosal integrity loss favours transmission.

2. Occurrence and incidence

Natural TVT frequently has been seen in the external genitalia (Rogers *et al.*, 1998) [39], Novinsky was successful in experimentally transplanting this tumor. The primary ocular masses developed first from the conjunctiva in 1876 (Murgia *et al*., 2006) [31]. It is evident mainly with significant populations of free-roaming dogs with suboptimal breeding practices (Papazoglou 2001, Boscos 2004) [35, 8].

Although there is no particular predisposition to age, gender or breed, yet large breeds are frequently affected more (Das 2000, De Lorimier 2007) [10, 11] but maybe transmitted through licking, biting, and sniffing tumor affected areas. It is enzootic in areas with poor breeding controlled and high numbers of free-roaming sexually active dogs (Das and Das, 2000) [10]. Extragenital primary TVT is rare, while the most common sites are usually the nasal and oral cavities, skin, and rectum spread by sniffing or licking or biting the genitalia of carrier animals are also reported (Mukaratirwa 2003, Veloso 2018) [30, 41]. Commonly transmission occurs during coitus through injured mucosa by viable tumor cells (Das and Das, 2000) [10]. The regional lymph node is the most common site of metastasis (Baştan 2008) [7], genital TVT proliferation is cauliflower-like with ulceration, while extra genital TVT is generally in the nodular form with multiple lobules, variable sizes of firm ulcerative mass or solitary nodule having potency to invade the mucosa and submucosa (Gurel, 2002) [16].

However, cutaneous metastasis is more evident in males than in females (Boscos and Ververidis, 2004) [8]. The metastasis rate of 1.5 to 6% (Rogers 1997) or 0 to 17% (MacEwen (2001) was observed in naturally occurring TVT. Multiple organ metastases are seen in TVT, besides subcutaneous, such as lung, spleen, kidneys, anterior mediastinum, liver, and in thoracic and abdominal cavity superficial and deep lymph nodes of the (Park *et al*.., 2006). Rare cases of TVT also seen in the adenohypophysis (Manning and Martin, 1970) pharynx, tongue, (Ndiritu *et al*.., 1977), and even the brain (Kroger *et al*.., 1991) [21]. According to Central Toronto Veterinary Referral Clinic, TVT is typically a slow-growing cancer with a shallow metastatic rate, reported being between 5% and 17%. The base of penis which is only seen on complete retraction of sheath is most common in males. No breed or sex predilection has been noted.

TVT is prevalent in 2 to 5 years age. They are easily transmitted within a household by a new dog, even if other dogs in the home are neutered or spayed. Transmission can occur quickly in either direction between the dog and the bitch. Clinical evaluation of the dogs revealed mild but progressive anorexia, dehydration, and polydipsia, high infiltration of T lymphocytes is seen in regressing tumors (Hill *et al*.., 1984) [17] inducing regression by cellular differentiation (Yang *et al*., 1991) [44]. Risk factors for TVT in bitches (Aydın *et al*.., 2009) [5] are mostly malnutrition and oxidative stress.

3. Diagnosis

- Fine needle aspiration cytology (FNAC) is done using 23-25 G needle and 2-5 mL syringe before surgical resection (Thangumalai, 2008) variation in the cellular (anisocytosis) and nuclear morphology (anisokaryosis) was prominent. The nucleus is centrally placed (Thangumalai, 2008). The cytoplasm appeared grayish on staining with Romanowsky in combination with Giemsa stains. while cells, when stained with hematoxylin and eosin (H&E), were basoeosinophilic.
- Based on physical examination and cytological findings, definitive diagnosis is made. (Kroger1991) [21]. Since cytology is minimally invasive and painless, it is the best choice for diagnosis along with being simple and cheap produces much less distortion of cell morphology is caused by cytology than formalin-fixed biopsy samples (Amaral 2007) [12].
- Wet fixation smears were stained with Harris Hematoxylin and Eosin (Bancroft and Stevens, 1996) [6]. However, Wright-Giemsa (WG), Wright's (W), May-Grünwald-Giemsa (MGG) and Leishman-Giemsa (LG) were used for the air-dried smears. Impression smears can be prepared from different areas of the tumor masses. Fixation of smears made is done either by wet fix with absolute isopropanol or 95 per cent ethanol (Allen *et al*.., 1986) [1] for 20 min or air-dried immediately. The occurrence of individual round cells in a branching fibrovascular network helps in the histological diagnosis of the tumor. (Krithiga *et al*.., 2005; Maclachlan and Kennedy, 2002) [20, 24].
- R. Thangathurai *et al*.. 2008) [40] suggested that diagnostic cytology is only method for detecting TVT. Polycythemia in blood is highly evident in animals suffering from TVT. This may be diagnostic with validation.

4. Histopathology

- TVT is classified into atypical and typical (this is inadequate data determinate cell type prevalence is different in different areas). TVT is plasmacytic. TVT of lymphocytic morphology predominates (non-published data).
- Two morphologically distinct categories of TVT cells in *in vitro* were described by (Mohanty and Rajya (1977). The Veterinary Pathology Service of FMVZUNESP, Botucatu, Brazil, adopted the classification of TVT based on cell morphology since 1994. Large round cells with a round nucleus, coarse chromatin, one to two prominent nucleoli, abundant and lightly basophilic cytoplasm, and multiple punctate vacuoles are characteristic features of TVT cells. (Kroger1991) [21] The high mitotic index and the numerous, well-demarcated intracytoplasmic vacuoles characteristically differentiate from other more common round cell skin tumors (de Lorimer 2007) [11] on observation under the microscope, a stained section with Masson´s Trichrome, sheet or rows of tumor cells was evident with scanty blue fibrous stroma (Ayyappan *et al*.., 1994) [4]. macrophages, lymphocytes, and plasma cells infiltration in tumor cells along with intense fibroblastic proliferation and collagen deposition give evidence of scirrhous reaction (Nak *et al*.., 2005) [32].
- Mitotic figures were prominent in different stages of mitosis, indicating the proliferative tumor cells. Many workers reported comparable cytological features (Duncan and Prasse, 1979; Fan *et al*.., 2001) [13, 14].Variation in the nuclear morphology (anisokaryosis) and cellular (anisocytosis) are prominent observations. Molecular markers like proliferating cell nuclear antigen (PCNA), AgNOR, etc. for differentiation are being focused as recent approaches. Studies indicated the differences in cell types for the progression of TVT. Progressive growth stage tumor typically has round cells with microvilli, whereas a regressing tumor exhibits

transitional, rather fusiform cells. Furthermore, a high number of T-lymphocytes are found in regressing tumors. (Hill *et al.*, 1984) [17] cytological findings of TVT are collected from exfoliated cells obtained by swabs, fine needle aspiration, or imprints of the tumors (Moulton, 1978) [29]. TVT displays histological resemblance to canine cutaneous histiocytomas and other round cell tumors, thereby presenting great difficulties for pathologists in their differentiation (Pawaiya *et al.*, 2006) [36].

- The examination must be done under a light microscope after staining with hematoxylin and eosin (H and E) and Masson´s Trichrome. The immune reactions is done (Miettinen *et al.*, 2000) [28] using the avidin-biotin-peroxidase technique (Dako, Carpinteria, CA, USA). The chromogen used Diaminobenzidine (DAB) to visualize the immune stain. Monoclonal mouse Vimentin is the primary antibodies for study (#M0725, Dako) besides rabbit polyclonal S100 (#Z0311, Dako).
- Localized antibody-mediated control of TVT is characterized by the occurrence of the significant count of lymphocytes, plasma cells, and activated macrophages (Mascarenhas *et al.*, 2014). Comparatively high nucleus: cytoplasm ratio is observed. Generally, TVT is multicellular and contains round or oval cells that vary between 14 and 30 µ in diameter, with well-demarcated cytoplasmic borders. Plasmacytic TVT is more aggressive than the lymphocytic form according to Amaral, 2005; Gaspar, 2005; Bassani-Silva, 2007. (Mohanty and Rajya 1977) explained the two morphologically distinct types of TVT cells *in vitro*: one, large cells with a hyperchromatic nucleus and acidophilic cytoplasm.; and the other, small cells with round to oval nucleus, Immune-mediated control is suggested by frequent an infiltration of lymphocytes, plasma cells and macrophages. TVTs should be distinguished from mastocytomas, histiocytomas, or malignant lymphomas.
- Due to different cell lineages of TVT, varying degrees of aggressiveness with variable biological behavior is observed. The cellular aspect can be either the primary tumor along with metastasis, or can be atypical as in cases of old tumors, Murgia *et al.*. (2006) [31], detected sequential differences between the tumors after analyzing an amplified mitochondrial DNA fragment. Based on this, the tumor is divided into two subclasses, as plasmacytic morphology and lymphocytic or mixed morphologies based on an ancestral clone. Plasmacytic tumor exhibiting higher frequency of nuclear abnormalities, are associated with greater expression of glycoprotein-P and amplified resistance to antitumoral action of propolis with almost all cases of metastasis being of the plasmacytic type. It is concluded that this type is rather aggressive, i.e., more malignant compared to those of lymphocytic or mixed morphologies.
- Histology revealed compact masses or confluent sheets of cell growth although they might branch in rows, cords, or loose in a delicate stroma, the cells converted to irregular shape tightly packed and in and fibroblasts appear, with the increase in tumor mass indicating the transformation of tumor cells.

5. Treatment

Chemotherapy, surgery, radiotherapy, and immunotherapy are the various treatment protocols for TVT (Pigatto, 2011) [37]. irrespective of the neoplasm size, extent, and duration of the disease, intravenous administration of Vincristine once a week, at the dose of 0.6 mg/m2 to 0.8mg/m2 of the body surface, for 2-6 weeks, is the best treatment of choice (Rani, 2015) [38] such as gastrointestinal effects, myelosuppression, are Side effects of Vincristine along with peripheral neuropathy paresis in around 5 to 7% of the patients (Rani, 2015) [38] extravasation of the drug caused local tissue reactions (Pigatto, 2011) [37]. Vincristine triggers the regression of the tumorous nodules due to the mitotic arrest of the tumor cells (Nak *et al.*, 2005) [32].

The possible explanation for high recurrence rate conceivable due to incomplete excision of the tumor nodules following surgery owing to the inaccessibility of the tumor sites, besides metastasis. tumor transplantation into the surgical wound by with 30-75% surgery recurrence rate contaminated instruments or gloves pose the post-operative threat of recurrence while the marginal surgical excision is not sufficient (Martins *et al.*, 2005) [25] (Idowu, 1984) [18].

Potential treatments of TVT include surgical removal of neoplastic nodules (Kunakornsawat *et al.*, 2010) [22], radiotherapy (Rogers *et al.*, 1998) [39] or chemotherapy with vincristine sulfate (Amber *et al.*, 1990) [3], and most recently using interleukin 2 (IL2) (Otter *et al.*, 2015) [33, 34]. Chemotherapy with vincristine sulfate is the most effective protocol for TVT treatment (Otter *et al.*, 2015) [34, 35]. in Vincristine resistant tumors, doxorubicin is the drug of choice. TVT is singular in its sensitivity to a variety of treatments.

Chemotherapy doesn't lead a favorable response in senile tumours (Boscos *et al.*, 1999) while growing tumors responded well to an early diagnosis being related to morphology modifications.

Some TVT shown tolerance to chemotherapy upon numerous administrations. Multidrug resistance was due to P-glycoprotein (P-GP) emergence, which is the main factor responsible for the emergence of a transporter protein encoded by the MDR1 gene that exists in healthy tissues (CNS, intestinal cells, renal tubular cells, and bile canaliculi) and tumor tissues. The P-GP is a substrate for various molecules, involving an efflux of substances from within the cell.

It acts as a crucial drug efflux pump hence excreting drugs like Vincristine (Korystov *et al.*, 2004) and other drugs such as vinblastine, doxorubicin, ivermectin and loperamide (Mealey *et al.*, 2003) rather than absorbing in the body.

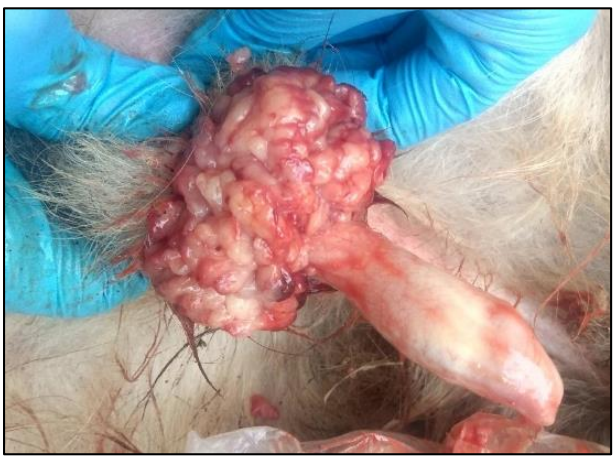

Fig 1: A case of male TVT before any treatment on retraction of the sheath

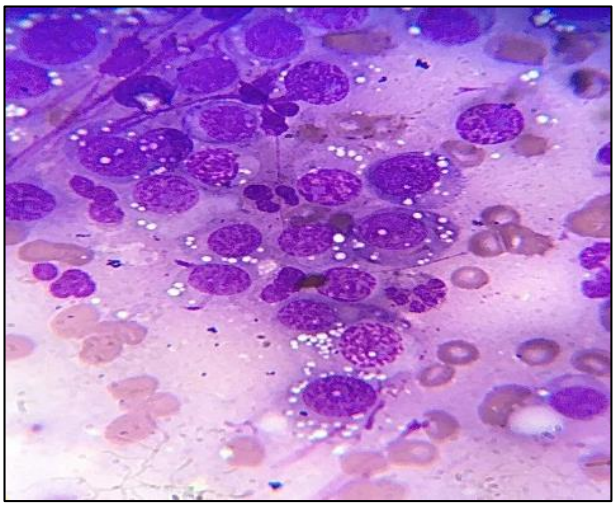

Fig 2: vacoulated plasma cells observed under microscope (H & E 100)

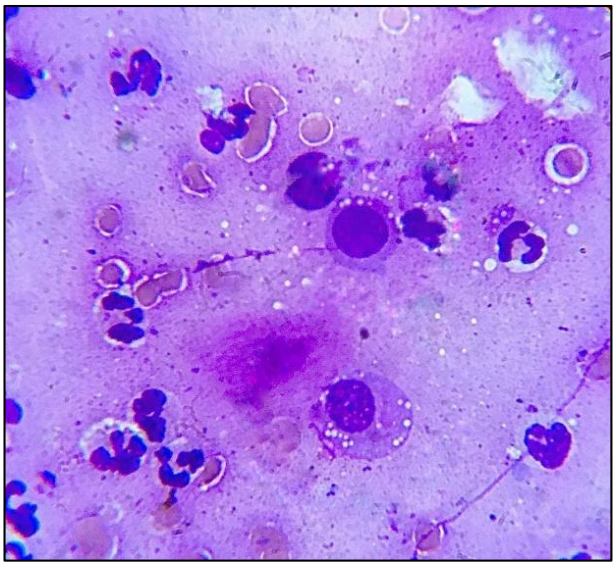

Fig 3: Vacoulated plasma cells under microscpoe from the male TVT case (H & E 100)

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