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RESEARCH ARTICLE

Clinico-Pathological Evaluation and Treatment Outcomes of Canine Transmissible Venereal Tumor Using Three Different Protocols

Mohamed Fathi[1], Mohamed Ashry[1,2*], Khaled M. Ali[3], Azza Hassan[4] and Amr F Elkarmoty[5]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[2]Laboratory of Mammalian Reproductive Biology, Department of Animal Science, Michigan State University, East Lansing, MI 48824, USA; [3]Department of Surgery, Anesthesiology and Radiology; [4]Department of Pathology; [5]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
*Corresponding author: ashry@cu.edu.eg

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ABSTRACT

The objectives of this study were to demonstrate the clinical and pathological findings of TVT, and to investigate the outcomes of three management protocols including chemotherapy with vincristine, surgical intervention and combination of surgery and chemotherapy under field conditions in Egypt. This study was conducted on 104 dogs of different ages and breeds (Male=51, Female=53). Diagnosis of TVT was evident by gross clinical manifestations, ultra-sonographic, and histopathological examination. Dogs were allocated to 3 groups. Group 1 (n=36), was treated by chemotherapy with IV injection of vincristine sulfate for 4 weeks, complete regression of the tumor nodules was observed in 29 cases (80.55%), while 7 cases (19.45%) had incomplete regression at the end of 4 weeks, group 2 (n=34), was treated by surgical de-bulking of the tumor nodules, complete regression was observed in 19 (55.88%) whereas 15 cases (44.12%) showed incomplete regression and recurrence of the tumor nodules and group 3 (n=34), was subjected to surgical intervention followed by vincristine treatment, 31 dogs (91.17%) showed complete regression with only 3 cases (8.83%) were not completely recovered, suggesting that surgical treatment coupled with vincristine chemotherapy is the most efficient protocol of TVT treatment in dogs.

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INTRODUCTION

Canine transmissible venereal tumor (TVT), also known as Sticker's sarcoma is a contagious, naturally occurring, horizontally transmitted venereal round cell tumor (Murgia *et al.*, 2006). It is normally transmitted during coitus by viable tumor cells through injured mucosa, but may be transmitted through licking, biting, and sniffing tumor affected areas (Das and Das, 2000). In enzootic areas, where breeding is poorly controlled and there are high numbers of free-roaming sexually active dogs, TVT is the most common canine tumor (Das and Das, 2000; Ganguly *et al.*, 2016). The lesions of TVT are usually confined to the mucous membranes of the external genitalia of dogs of both sexes of any breeds (Amaral *et al.*, 2004). Usually, TVT lesion remain localized, but metastasis to the adjacent skin, oral, ocular, nasal and conjunctiva mucosae and inguinal lymph nodes was reported in many cases (Chikweto *et al.*, 2013; Milo and Snead, 2014; Komnenou *et al.*, 2015). Cutaneous TVT without genital involvement was also reported in a prepubertal female dog (Marcos *et al.*, 2006). Initially the tumor lesion is small, subsequently progressing to a large, ulcerated, and contaminated mass (Das and Das, 2000). The lesions are friable, hyperemic, multi nodular, cauliflower like masses and the accompanying hemorrhagic discharge produces offensive odor (Do Amaral *et al.*, 2007).

Despite of its malignant potential, the responsiveness of TVT to a variety of treatments is remarkable. Possible treatments of TVT include, surgical de-bulking of the neoplastic nodules (Kunakornsawat *et al.*, 2010), chemotherapy with vincristine sulfate (Amber *et al.*, 1990), radiotherapy (Rogers *et al.*, 1998) and most recently using interleukin 2 (IL2) (Otter *et al.*, 2015). Chemotherapy with vincristine sulfate is an effective protocol for TVT treatment (Otter *et al.*, 2015). Therapy with four to eight

intravenous doses is being necessary to obtain an acceptable result (Erunal-Maral *et al.*, 2000).

The objectives of the current study were to demonstrate the clinical and pathological findings of TVT, to investigate the outcomes of three treatment protocols including chemotherapy with vincristine, surgical intervention and combination of surgery and chemotherapy and to record the prevalence of TVT infection among dog breeds in Egypt.

MATERIALS AND METHODS

Animals: This study was carried out on 104 dogs of different ages (2-8 years old), body weights and of both sexes (Male=51, Female=53). The breeds included in this study were Great Dane (54), German shepherd (38), Boxer (7) and Pitbull (5). These dogs were brought, to Theriogenology clinic, Faculty of Veterinary Medicine, Cairo University and other private pet animal clinics in Cairo, with a history of hemorrhagic discharges, difficult breeding and, in some cases, presence of nodular mass within and around the external genitalia. Initial investigations showed gross anatomical distortion where, the male dogs had nodular lesions on the penis and the preputial mucosa, while the bitches had the lesions extending into the vagina, heavily swollen and pendulous vulva, with serosanguinous fluid exuding from the vulva. Data were collected from the clients including duration of clinical signs, and history of any previous medications or intervention. The affected dogs were allocated into 3 groups according to the treatment protocol applied.

Ultrasonographic examination: Ultra-sonographic examination was performed on females when the nodules were not visible externally; the swollen vagina was examined externally using 6MHz trans-abdominal transducer attached to ultrasound machine (SonoScape, Model A6 - China) and aimed the detection of the size of the nodules and the extent of vaginal occlusion.

Histopathological examination: Different random specimens from tumor masses were fixed in 10% neutral buffered formalin, washed, dehydrated and embedded in paraffin. Five µm-thick sections were stained with hema-toxylin and eosin (H and E) and Masson´s Trichrome and examined under light microscope (Sales Lapa *et al.*, 2012).

Immunohistochemical analysis: The immune reactions were performed as described by (Miettinen *et al.*, 2000) using the avidin biotin peroxidase technique (Dako, Carpinteria, CA, USA). Diaminobenzidine (DAB) was used as the chromogen to visualize the immune stain. The primary antibodies used in this study were monoclonal mouse Vimentin (#M0725, Dako) and rabbit polyclonal S100 (#Z0311, Dako).

Treatment protocols: Group 1: Dogs (n=36) were treated by chemotherapy using vincristine sulfate only Vincristine®, Rex pharma, Hungary) as 0.01% solution, at a dose rate of 0.025 mg/kg body weight by slow intravenous injection (IV). The treatment began once the diagnosis was confirmed and repeated weekly for 4 weeks, after the end of the fourth week animals were subjected to surgical removal of any persistent tumor masses.

Group 2: Dogs (n=34) were treated by surgical removal of the tumor nodules. All surgeries were performed under general anesthesia using Xylazine (1 mg/kg body weight "bw", Adwia, Egypt) and Ketamine hydrochloride (Protexmedica, Trittau, Germany) at dose of 15 mg/kg bw. After preparation of the dogs for aseptic surgery, the technique involved surgical de-bulking of the nodules and the complete dressing afterwards. In some cases, when the nodules were occluding the vagina, application of a Foley catheter through the external urethral orifice was used.

Group 3: Dogs (n=34) were subjected to surgical removal of the tumor nodules coupled with chemotherapy by Vincristine sulfate (0.025 mg/kg bw IV). The first dose of chemotherapy was administered at the same day of surgical de-bulking and repeated weekly for 4 weeks.

Response to treatment: Response to treatment protocol was evaluated on basis of regression of the nodules after 4 weeks of treatment. Side-effects or unwanted sequels of treatments or surgical intervention, if any were recorded.

Statistical analysis: Treatment response data were analyzed by analysis of variance (ANOVA) using the general linear models' procedure of SAS (SAS Institute Inc., Cary, NC). Percentage data was arcsine transformed before analysis.

RESULTS

Anatomical and ultra-sonographic evaluation: The initial gross anatomical investigation of the dogs affected by TVT showed easily bleed, friable, nodular, cauliflower like masses with an offensive odor located on the preputial mucosa and on the penis in males (Fig. 1). In females, these lesions were located in the vicinity of vagina and mucosa of the vulva and were accompanied by serosanguinous discharges with offensive odor (Fig. 2). Interference with copulation and/or bleeding after copulation was the most problem reported by the owners. Metastasis to the skin of the back region was recorded in three cases (2.88%) with subcutaneous movable nodules lacking the hair over (Fig. 3). Ultra-sonographic imaging revealed the nodular lesions of variable sizes and of mixed echogenicity; hyperechoic echoes were identified at the center of the nodules and less echogenic echoes were at the periphery of the nodules (Fig. 4a-b). Phimosis was only reported in two males with large nodular lesions located on the tip of the penis and the anterior part of the preputial mucosa (anatomical distortion).

Treatment response: Response to each treatment protocol was evaluated based on regression and disappearance of the tumor lesions after 4 weeks of treatment (Table 1). Group 1 (n=36) was treated by chemotherapy with IV injection of Vincristine sulfate for 4 weeks, complete regression of the tumor nodules was observed in 29 cases (80.55%), while 7 cases (19.45%) had incomplete regression at the end of 4 weeks. In group 2 (n=34), treated by surgical de-bulking of the tumor nodules under general anesthesia, complete regression was observed in 19 (55.88%) whereas 15 cases (44.12%) showed incomplete regression and recurrence of the tumor nodules. In group 3 (n=34), subjected to surgical intervention followed by Vincristine treatment, 31 dogs

(91.17%) showed complete regression with only 3 cases (8.83%) were not completely recovered. Results indicated that both treatment with chemotherapy and surgical removal of the tumor combined with chemotherapy resulted in significant treatment response ($P<0.05$) compared to surgical removal only. Although there was no significant difference between the response to chemotherapy alone and surgical treatment coupled with Vincristine chemotherapy, the later tend to have higher response ($P=0.06$), suggesting that surgical treatment coupled with vincristine chemotherapy is the most efficient protocol of TVT treatment in dogs. Surgical de-bulking of the nodules was very efficient in males (Fig. 5).

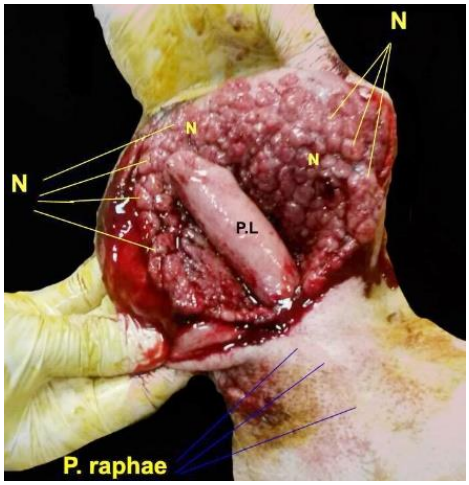

Fig. 1: Intra operative photograph showing multiple nodular lesions (N) on the preputial mucosa of a 3 years old male German shepherd. PL; pars longus of penis, P; penile.

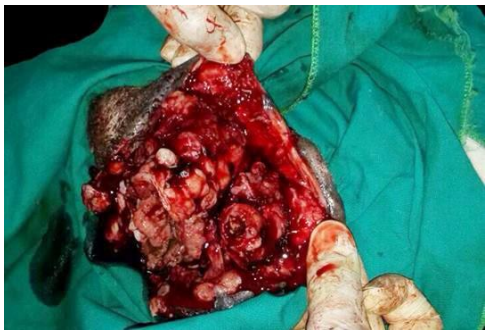

Fig. 2: Intra operative photograph showing cauliflower like nodules with serosanguinous discharges occluding the vagina of 2 years old Pitbull bitch.

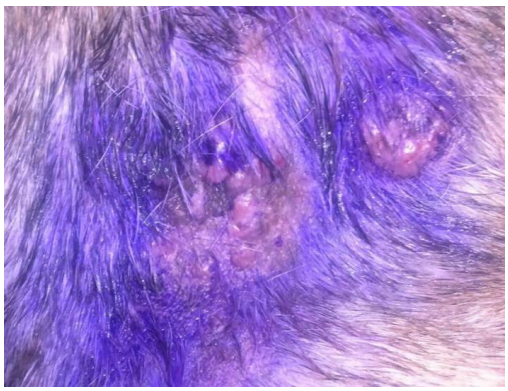

Fig. 3: A photograph showing tumorous growths on skin of the back region in 8 years old male Great Dane. The lesion was previously misdiagnosed, and the owner used to put Gentian violet as a topical treatment.

Histopathology and immunohistochemistry: The histopathological alterations revealed confluent sheet (Fig. 6a), or rows (Fig. 6b) of tumor cells separated by scanty fibrous stroma that appeared blue in Masson´s Trichrome stained section (Fig. 6c). The neoplastic cells were round to ovoid with large round to oval nuclei and prominent nucleoli and scanty cytoplasm in addition to frequent mitotic activity (Fig. 6d) that was demonstrated in all examined sections. Tumor cells were infiltrated by macrophages, lymphocytes and plasma cells with schirrous reaction characterized by intense fibroblastic proliferation and collagen deposition (Fig.7a-b). Some examined sections (4.16%) showed large area of tumor cell necrosis with fragmented nuclei (Fig. 7c)**.** Immunohistochemical examination of tissue sections revealed tumor cells strongly and diffusely positive for Vimentin (Fig. 7d and 7e), whereas completely negative for S-100 (Fig. 7f).

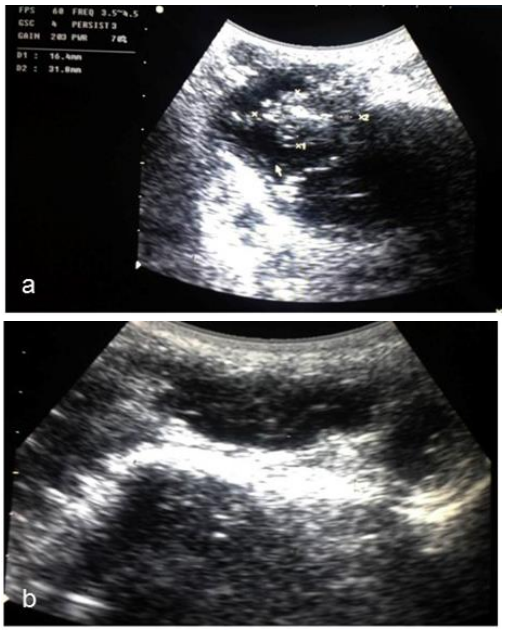

Fig. 4: (a) Transverse sonogram on the vagina of a 2 years old female boxer showing nodular mass (3.1 cm x 1.6 cm) with mixed echogenicity occluding the vaginal lumen, (b) Transverse sonogram on the vagina of a 3 years old female Great Dane showing nodular mass with mixed echogenicity partially occluding the vaginal lumen.

Fig. 5: Intra operative photograph showing clear preputial mucosa (PM) after surgical removal of the nodular lesions in a 3 years old male German shepherd.

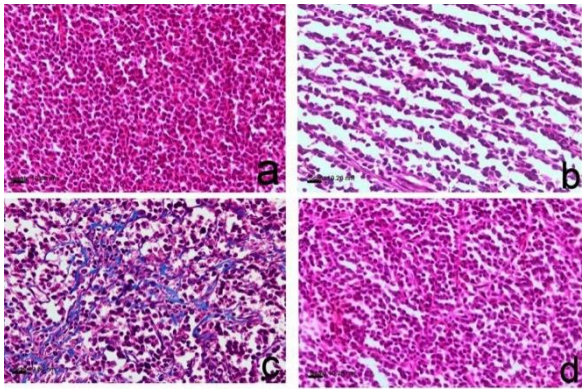

Fig. 6: Tissue sections of tumor masses showing (a) confluent sheet of tumor cells separated by scanty fibrous stroma (HE, 20µm), (b) tumor cells arranged in raw (HE, 20µm), (c) blue scanty fibrous stroma (Masson´s Trichrome, 20µm), (d) frequent mitotic activity (HE, 20µm).

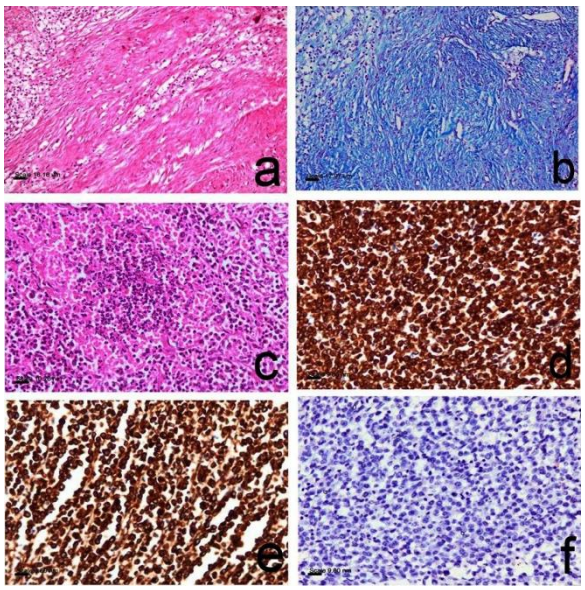

Fig. 7: Histological sections of tumor masses showing (a) intense collagen deposition (HE, 20µm), (b) blue stained collagen fibers (Masson´s Trichrome, 20µm), (c) tumor cell necrosis with fragmented nuclei (HE 20µm), (d & e) tumor cells strongly and diffusely positive for Vimentin (IHC, 20µm), (f) tumor cells completely negative for S-100 (IHC, 20µm).

Table 1: The clinical outcomes for different treatment protocols

Treatment protocol	Number of cases	Recovered cases	
		Number	%
Group 1: chemotherapy: (Vincristine sulfate, 0.025 mg/kg IV)	36	29	80.55[a]
Group 2: surgery	34	19	55.88[b]
Group 3: surgery followed by chemotherapy	34	31	91.17[a]

Different superscripts in the same column are significantly different at $P<0.05$.

DISCUSSION

Dogs have become an important household pet in Egypt. Both the number of dogs and pet animal clinics are exponentially increasing over the last few years in Egypt. It has been reported that TVT affects dogs of any breeds, age or sex with high incidence during the periods of maximal sexual activity; estrus in female (Kabuusu *et al.*, 2010). To our knowledge, management of TVT has not been investigated under clinical field conditions in Egypt. Therefore, the current studies investigated the treatment outcomes of three different protocols in Egyptian dog population affected by TVT.

In the current study, higher incidence of TVT infection were recorded for Great Dane (54 cases; 51.92%) and German shepherd (38 cases; 36.53%) of the reported cases. This may be affected by the breed ownership preference in Egypt. Canine TVT is most common in dogs of 2-5 years (Das and Das, 2000). In the present study, dogs were 2-8 years. Malnutrition and oxidative stress have been incriminated as risk factors in dogs with lymphoma (Winter *et al.*, 2009) and TVT in bitches (Aydın *et al.*, 2009).

Exfoliation and transplantation of neoplastic cells occurs during mating or licking of affected genitalia providing the main mode of transmission to the genital mucosa, and also into nasal or oral mucosa (Amaral *et al.*, 2004). TVT usually affects the external genital organs, but presence of TVT lesion were reported in the skin, nasal mucosa, oral mucosa, eye, liver, lung, spleen, brain, uterus, ovary and mammary glands (Varughese *et al.*, 2012; Milo and Snead, 2014; Komnenou *et al.*, 2015; Rezaei *et al.*, 2016). Cutaneous metastasis is more frequent in males than females (Boscos and Ververidis, 2004). In the present study, the lesions of TVT in dogs were confined to the penis and preputial mucosa whereas; in females, the lesion was noticed on and in the vagina, vestibule, clitoris and vulva. Cutaneous metastasis was observed in 3 cases (2.88%), the lesions were scattered nodules over the back and flank and subcutaneous movable nodules lacking the hair over. however, the present study did not investigate abdominal metastasis, therefore metastasis of internal organs cannot be ruled out.

Interference with copulation and/or bleeding after copulation caused by destruction of the friable nodules inside the vagina, were the most common problems reported by the owners. In some cases, where the nodules were not visible externally in the females, the ultrasonography can be utilized as an effective tool to determine the size, location of the nodules and the extent of vaginal occlusion. In the current study, ultrasonographic diagnosis revealed the lesions of TVT as masses with mixed echogenicity; hyperechoic echoes were identified at the center of the mass and the less echogenic echoes were at the periphery which may be due to the fibrous nature of the tumor and to the schirrous reaction caused by the intense fibroblastic proliferation and collagen deposition.

The histopathological examination which confirmed the diagnosis was congruous with a previous study which demonstrated the tumor as confluent sheet or rows of tumor cells separated by scanty fibrous stroma that appeared blue in Masson´s Trichrome stained section (Ayyappan *et al.*, 1994). Tumor cells were infiltrated by macrophages, lymphocytes, and plasma cells with schirrous reaction characterized by intense fibroblastic proliferation and collagen deposition (Nak *et al.*, 2005). The presence of large numbers of lymphocytes, plasma cells and activated macrophages in the tumor strongly suggests a role for localized antibody-mediated control of TVT (Mascarenhas *et al.*, 2014).

Several treatment protocols have been established for TVT, chemotherapy with vincristine sulfate is the most widely used protocol (Hantrakul *et al.*, 2014). It has been found to cause mitotic arrest of the tumor cells leading regression of the tumorous nodules (Nak *et al.*, 2005).

Cyclophosphamide and Methotrexate are chemotherapeutic agents that were previously used for treatment of TVT (Amber *et al.*, 1990). Chemotherapy with vincristine sulfate is associated with incomplete regression of the tumor nodules and recurrence in other cases. On the other hand, surgery can be an effective treatment for small localized TVT only; however, surgery has an overall recurrence rate of 30-75% and marginal surgical excision is not effective (Idowu, 1984). In addition, tumor transplantation into the surgical wound by contamination of instruments or gloves may also cause post-operative tumor recurrence (Martins *et al.*, 2005). Therefore, we hypothesized that combined treatment (surgery and chemotherapy) may improve the response to chemotherapy and reduce the recurrence rate. Our results demonstrated that surgical de-bulking of the tumor nodules followed by vincristine treatment was the most efficient treatment protocol. The recovery rate after 4 weeks following surgery combined with chemotherapy was 91.17% compared 88.55 and 55.88% in response to chemotherapy and surgical treatment respectively. The possible explanation for high recurrence rate after surgical intervention may be incomplete excision of the tumor nodules due to the inaccessibility of the tumor sites, and metastasis (Athar *et al.*, 2001). Therefore, the combined treatment protocol; surgery followed by chemotherapy is the optimal management protocol for TVT in dogs. It reduces the recurrence rate because of the action of vincristine on any tumor remnants that may be missed due any reason during the surgical procedures.

Conclusions: The present study demonstrated that combined surgical and chemotherapeutic treatment is the most effective protocol for management of transmissible venereal tumors in dogs under clinical field conditions in Egypt.

Authors contribution: MF, MA and KMA conceived and designed the study. MF and KMA executed the clinical study and performed the surgical procedures. AH performed the histopathological findings of the study. AFE described the anatomical distortion of the vagina or the penis caused by the tumor. All authors analyzed, interpreted and revised the data. KMA, MA and MF drafted the manuscript. All authors reviewed and approved the last version of the manuscript.

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