VETERINARSKI ARHIV 82 (4), 401-410, 2012

Clinico-pathological findings in a case of canine cutaneous metastatic transmissible venereal tumor

Suvendu K. Behera[1]*, Nitin P. Kurade[2], Shongsir W. Monsang[3], Durga P. Das[4], Krishna K. Mishra[1], and Ranjan K. Mohanta[5]

[1]Ph.D. Scholar, Division of Medicine, Indian Veterinary Research Institute, Izatnagar, India

[2]Senior Scientist, Division of Pathology, Indian Veterinary Research Institute, Izatnagar, India

[3]Ph.D. Scholar, Division of Surgery, Indian Veterinary Research Institute, Izatnagar, India

[4]Ph.D. Scholar, Division of Public Health, Indian Veterinary Research Institute, Izatnagar, India

[5]Ph.D. Scholar, Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar, India

BEHERA, S. K., N. P. KURADE, S. W. MONSANG, D. P. DAS, K. K. MISHRA, R. K. MOHANTA: Clinico-pathological findings in a case of canine cutaneous metastatic transmissible venereal tumor. Vet. arhiv 82, 401-410, 2012.

ABSTRACT

A metastatic form of cutaneous transmissible venereal tumor was diagnosed in a mongrel dog. The dog had non-fluctuating and ulcerating neoplastic nodules throughout the subcutis with a history of spontaneous regression and recurrence of new growths at different sites. A presumptive diagnosis was made based on fine needle aspiration cytology of neoplastic nodules and an impression smear of ulcerated lesions, which was confirmed by histopathology. Erythrocyte oxidant-antioxidant status revealed increased lipid peroxidation and decreased antioxidant enzymes, which might have been due to the direct influence of the tumor or by associated factors such as tumor necrosis and/or the inflammatory changes. Increased early apoptosis was observed in peripheral blood leukocytes. Treatment with Vincristin along with other supportive therapy yielded an unfavorable outcome due to the advanced stage of malignancy.

Key words: lipid peroxidation, antioxidant, venereal tumor, histopathology

Introduction

Canine transmissible venereal tumor (TVT), also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or Sticker tumor, is a canine reticuloendothelial tumor that mainly affects the external genitalia, i.e. the vagina in bitches and the penis and prepuce in males. It is a naturally occurring allograft, usually

*Corresponding author:

Dr. Suvendu Kumar Behera, Ph.D. Scholar, Division of Medicine, Indian Veterinary Research Institute, Izatnagar-243 122, Uttar Pradesh, India, Phone: +91 99 8850 2156; E-mail: drsuvendu.kumar@gmail.com

ISSN 0372-5480
Printed in Croatia

transmitted during coitus and mainly occurs in young, sexually mature animals. Metastases have been reported in the eyes, skin, brain, subcutaneous tissue, lymph nodes, tonsils, liver, spleen, oral mucosa, hypophysis, peritoneum, and bone marrow (PRASAD et al., 2007; SANTOS et al., 2008). There have been many reports of cutaneous disseminated TVT in dogs, but reports of the metastatic form of cutaneous TVT are very few. In addition, to the authors' best knowledge, the changes in erythrocytic oxidant-antioxidant status and apoptosis in peripheral blood mononuclear cells in this type of disease are the first of its kind which may help in a better understanding of the disease pathogenesis. The present study communicates a case of metastatic transmissible venereal tumor with its diagnosis, clinical presentation, hemato-biochemistry, oxidant/antioxidant status, apoptotic changes in peripheral blood leukocytes, therapeutic assessment and prognosis.

Case history

A 4-year-old male mongrel dog, weighing 13 kg, with a history of subcutaneous tumorous growths developed thirty days previously, presented with complaints of inappetance, loss of body condition, depression etc. Abnormalities noted on the initial physical examination revealed hard non-fluctuating neoplastic nodules (>35 in numbers) of approximately 1-5 cm in diameter throughout the subcutis (Fig. 1) mainly on the dorsal and ventral body plane, buccal mucosa, head, ventral neck, axillary regions, middle of the thighs, penis (Fig. 1), on the right and left flank and at the base of the ear. The growths were of variable size, ulcerating, bleeding, infected and with a history of spontaneous regression with recurrence of new growths at different sites (Fig. 1). There was generalized lymphadenopathy characterized by marked nodular enlargement of the lymph nodes.

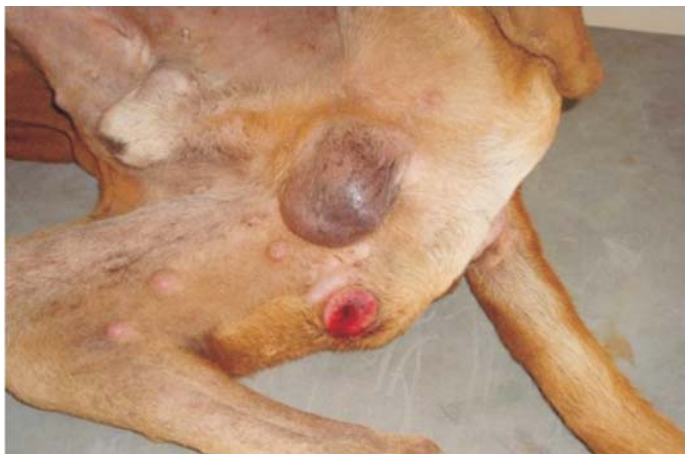
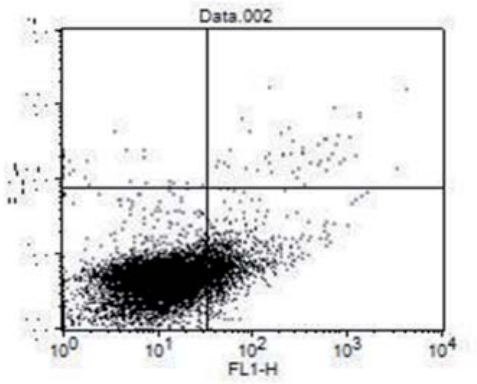


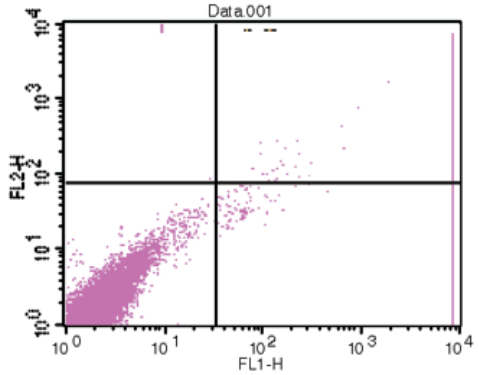
Fig. 1. Dog showing tumorous growths on penis and nearby areas.



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	28	0.28	0.28	5.76	2.88	159.04	143.58
UR	49	0.49	0.49	519.69	282.94	326.38	244.52
LL	8719	87.19	87.19	11.95	9.71	5.26	4.55
LR	1204	12.04	12.04	70.57	55.68	8.84	7.33

Fig. 2. Apoptosis in peripheral blood leukocytes of metastatic TVT.
Upper left quadrant: Necrosis (0.28%)), Upper Right quadrant: Late apoptosis (0.49%), Lower left quadrant: Healthy cells (87.19%), Lower right quadrant - Early apoptosis (12.04%)

A complete blood cell count on the day of presentation revealed normal Hb, hematocrit, total erythrocyte count and monocyte (Table 1). However, total leukocyte count was markedly elevated with neutrophilia, lymphopenia and thrombocytopenia. Serum chemistry was indicative of hypoproteinemia, hypoalbuminemia, hypoglobulinemia with higher levels of blood urea nitrogen and creatinine (Table 1). Increased activities of alanine aminotransferase and alkaline phosphatase (Table 1) were also observed. Oxidant-antioxidant status was estimated in the erythrocytes, which revealed a marked increase in the level of lipid peroxidation (LPO, 3.84 ηmol MDA/mg Hb; control: 0.94)**,** a decreased level of reduced glutathione (GSH, 0.76 µmol/mg Hb; control: 1.14), reduced activities of superoxide dismutase (SOD, 0.73 Units/mg Hb; control: 1.29) and catalase (CAT, 74.37 Units/mg Hb; control: 132.25). To demonstrate the effect of free-radicals on the immune system, the levels of apoptosis were measured in the peripheral blood leukocytes of both TVT positive and healthy animals using the Annexin-V EGFP apoptosis detection kit (GenScript, NJ, USA) by flow cytometry. The analysis revealed marked increase in early apoptosis (12.04%; healthy control: 0.74%), late apoptosis (0.49%; healthy control: 0.42%) and necrosis (0.28%; healthy control: 0.01%) (Fig. 2) in comparison to a healthy control (Fig. 3) indicating its role in the pathogenesis of the disease process.



Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1	0.01	0.01	1.00	1.00	763.51	763.51
UR	42	0.42	0.42	274.01	173.84	234.16	157.43
LL	9883	98.83	98.83	3.28	2.73	3.62	2.74
LR	74	0.74	0.74	72.95	67.15	44.70	42.76

Fig. 3. Apoptosis in peripheral blood leukocytes of healthy control.
Upper left quadrant: Necrosis (0.01%), Upper Right quadrant: Late apoptosis (0.42%), Lower left quadrant: Healthy cells (98.83%), Lower right quadrant - Early apoptosis (0.74%)

Fine needle aspiration cytology (FNAC) of the prescapular and popliteal lymph nodes and neoplastic nodules, an impression smear of ulcerated lesions along with the histological examination of the biopsied nodules, corroborated with the cutaneous lesions and growth on the penis (Fig. 1, 4-7) confirmed the diagnosis of primary cutaneous TVT. Ultrasound examination of the abdomen revealed metastases to the vital organs such as the liver, kidney and spleen.

Histopathology of the biopsied nodules revealed large, round or oval cells with indistinct outlines. The round or oval nuclei were very large in relation to the size of the cell. Mitoses were frequent. The stroma provided abundant blood vessels (Fig. 4) which were surrounded by inflammatory cells. The tumor cells were arranged in sheets or rows within the stroma of thin fibrous connective tissue (Fig. 5). FNAC and impression smear showed round or ovoid tumor cells containing single large round nuclei of variable size, coarse nuclear chromatin, large nucleoli and abundant vacuolated cytoplasm (Fig. 6-7).

Treatment constituted IV weekly Vincristin (Alkem, India), 0.025 mg/kg along with Ringer Lactate, 4.5 mL/kg/h IV and dextrose normal saline, 4 mL/kg/h with other supportive therapy like vitamin K1 (Injection Kapilin; GSK, India) 5 mg/kg BID, multivitamin (Injection Nurokind plus; Mankind, India) 4 mL IV SID.

Table 1. Hemato-biochemical observations in the mongrel dog

Parameters	Observed value	Reference range
Hemoglobin (g%)	12.4	12-18
Hematocrit (%),	40	37-55
Total erythrocyte count ($\times 10^6/\mu L$)	7.68	5.5-8.5
Total leukocyte count ($\times 10^3/\mu L$)	31.5	6-17
Neutrophils (%),	92	60-70
Lymphocytes (%)	4	12-30
Thrombocytes ($\times 10^3/\mu L$)	13.545	200-500
Monocyte (%)	4	3-10
Protein (g/dL)	4.2	6-8
Albumin (g/dL)	1.9	2.6-3.3
Globulin (g/dL)	2.3	2.7-4.4
Blood urea nitrogen (mg/dL)	39	8.8-26
Creatinine (mg/dL)	1.8	0.5-1.6
Alanine aminotransferase (U/L)	164	8.2-57
Alkaline phosphatase (U/L)	146	10.6-101

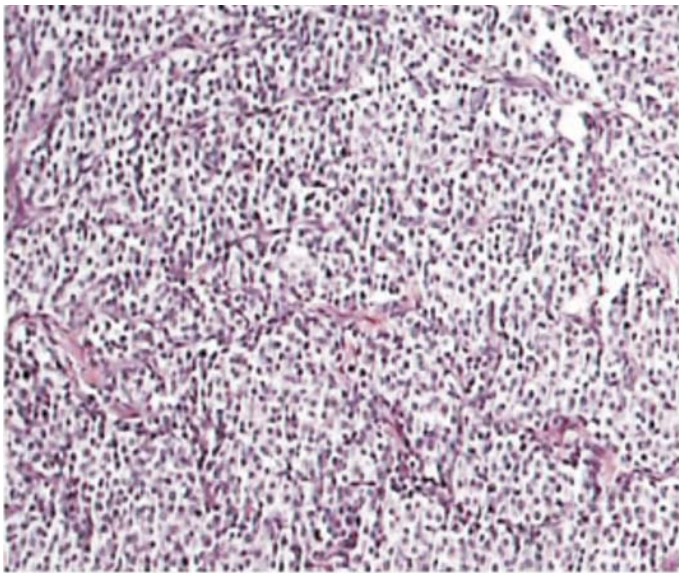


Fig. 4. Histopathology of biopsied nodule from the skin. Group of tumor cells arranged in sheets or rows within the stroma of thin fibrous connective tissue. HE; ×10.

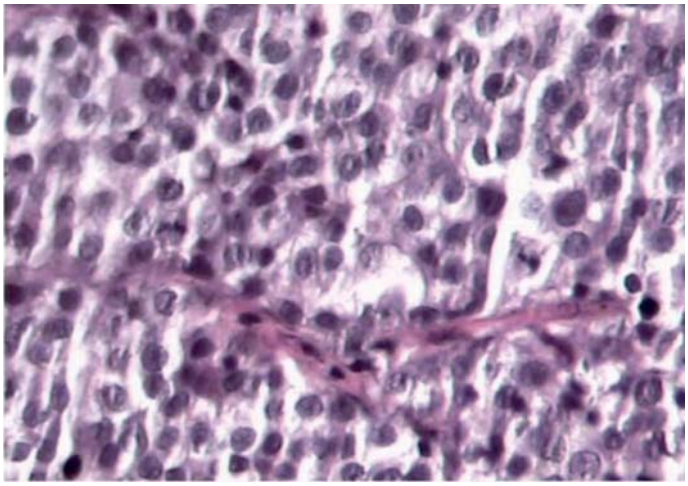


Fig. 5. Higher magnification of Fig. 4 showing cells with hyperchromasia, indistinct cellular outline, round nuclei separated by fibrous connective tissuue with numerous mitotic figures. H&E; ×40.

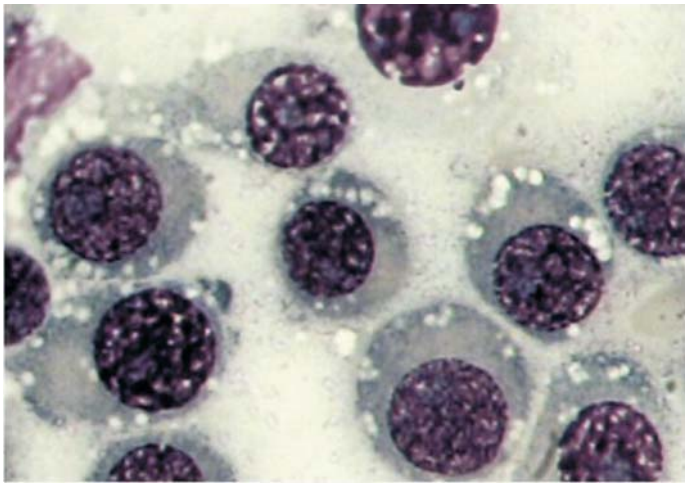


Fig. 6. Fine needle aspiration cytology of cutaneous TVT showing anisocytosis, round nuclei, large nucleoli and vacuolated cytoplasm. Giemsa stain; ×100.

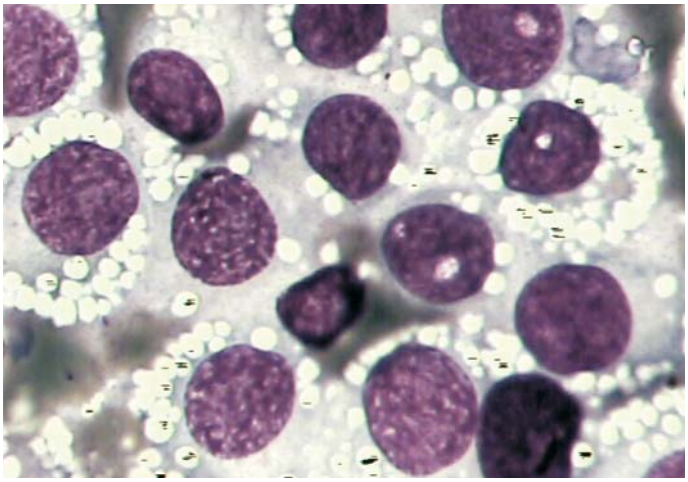


Fig. 7. Impression smear of ulcerated nodule. Sheet of cells with oval to round nucleoli and abundant vacuolated cytoplasm. Giemsa stain; ×100.

Discussion

TVT is a naturally occurring allograft (DAS and DAS, 2000). Exfoliation and transplantation of neoplastic cells occurs during mating or licking of affected genitalia. These physical contacts provide the main mode of transmission onto the genital mucosa, and also onto nasal or oral mucosa (COHEN, 1985). In the present case the cutaneous lesions appeared to be due to metastasis and dissemination of tumor cells. TVTs are immunogenic tumors and the immune system of the host plays a major role in inhibiting tumor growth and metastasis (COHEN, 1985). In young dogs (2-5 years) with maximum sexual activity or dogs with a compromised immune system, tumors have a greater tendency to metastasize and be virulent (DAS et al., 1991). Metastases were more frequent in males than in females (BOSCOS and VERVERIDIS, 2004). Our case fits the above mentioned risk factors which had a suboptimal physiological and malnourished status. Metastases to the visceral organs indicated the systemic and advanced state of the disease making the prognosis unfavorable (ODUYE et al., 1973).

Hematology was indicative of marked neutrophilic leukocytosis and lymphopenia owing to immunosuppression, leading to secondary bacterial invasion. The hematological findings were in contrast to DAS et al. (1991), who reported normal hemogram in canine TVT. This might be due to the advanced stage of the disease at the time of presentation. Hypoproteinemia and hypoalbuminemia were due to malnutrition and wasting condition in conjunction with the disease. There was a marked increase in the levels of ALT, ALP,

BUN and creatinine, probably due to metastasis to these organs affecting the organ function.

Free radicals are highly reactive molecules produced during normal metabolism in the body, or after exposure to environmental factors, which are kept in equilibrium by the body through endogenous antioxidant defense mechanisms (comprising of SOD, CAT, glutathione peroxidase, glutathione reductase and GSH etc.). If this balance is disturbed, it may result in oxidative stress, leading to enhanced lipid peroxidation, DNA strand breaks and protein damage (BEHERA et al., 2011). The role of oxidative stress in carcinogenesis has been delineated in dogs with lymphoma (WINTER et al., 2009), TVT in bitches (AYDIN et al., 2009) and canine mammary tumors (KUMARAGURUPARAN, 2005). LPO in the form of malondialdehyde (MDA), SOD and CAT were estimated in 10% hemolysate, whereas GSH was estimated in RBC suspension (1:1). In this case, MDA level, which is a sensitive indicator of LPO occurring under oxidative stress, was found to be markedly increased in comparison to a healthy control whereas the level of GSH and the activities of SOD and CAT were markedly reduced, indicating the role of free radicals in this disease. Oxidative injury to the erythrocyte membrane leads to increased lipid peroxidation in TVT because of infiltration of leukocytes into malignant tissues, producing ROS (PACHER et al., 2007). GSH protects -SH groups of lipoproteins present in the cellular membrane and hemoglobin from oxidative damage (FANG et al., 2002) and is also a good scavenger of many free radicals. SOD mainly acts by quenching superoxide and CAT by catalyzing the decomposition of H_2O_2 to water and oxygen. Lower levels of GSH and reduced activities of SOD as well as CAT may be explained by the overproduction of ROS, exhausting the body's self-correcting mechanisms to counter oxidative damage due to malignancy. Depletion of endogenous GSH has been associated with increased susceptibility of the cells to oxidative stress-induced apoptosis (GOEL and KHANDUJA, 1998). Our findings are in agreement with reports of enhanced lipid peroxidation and decreased antioxidant activity in different cancer types of humans (JEONG et al., 2009; BADJATIA et al., 2010) as well as canine transmissible venereal tumour (AYDIN et al., 2009). However, KUMARAGURUPARAN (2005) reported increased levels of both lipid peroxidation and antioxidant activity in a canine mammary tumor. The alterations in oxidant-antioxidant status might be due to direct injury by tumor cells or inflammation and/or necrosis which needs further validation in similar types of cases.

In the early stages of apoptosis, changes occur on the cell surface such as translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer, by which PS becomes exposed at the external surface of the cell. Annexin V is a Ca^{2+} dependent phospholipid-binding protein with high affinity for PS. The expression of PS on the cell surface, measured by Annexin V conjugated with an enhanced green fluorescent protein, was indicative of early apoptosis, whereas incorporation of propidium

iodide, a nucleic dye, was indicative of middle/late apoptosis (necrosis). The finding was consistent with GUVENC et al. (2002), but contrary to SHILPA et al. (2007) who denied any role of apoptosis contributing to spontaneous regression in TVT. Oxidative stress-induced apoptosis results in the oxidation of membranes, disruption of metabolic processes and alteration of certain proteins and transcription factors (GOEL and KHANDUJA, 1998) aggravating the condition. Inspite of all the recommended therapies the animal succumbed to malignancy after the 2nd dose of vincristin on day 10 post therapy. Necropsy of the animal could not be accomplished with respect for the sentiments of the owner.

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Received: 7 July 2011
Accepted: 17 April 2012

BEHERA, S. K., N. P. KURADE, S. W. MONSANG, D. P. DAS, K. K. MISHRA, R. K. MOHANTA: Peroksidacija lipida i antioksidativni status u psa mješovite pasmine s kožnim metastazama prenosivoga veneričnoga tumora - prikaz slučaja. Vet. arhiv 82, 401-410, 2012.

SAŽETAK

Metastaze kože prenosivoga veneričnoga tumora dijagnosticirane su u psa mješovite pasmine. Ustanovljeni su nefluktuirajući i ulcerativni neoplastični čvorovi u potkožju, koji su spontano nestajali, ali uz pojavu novih na različitim mjestima. Sumnja na pojavu tumora bila je postavljena na osnovi citološkoga nalaza aspiriranoga tkiva novotvorina, a potvrđena histopatološkom pretragom materijala uzetog iz ulceracijskih promjena. Antioksidacijski status eritrocita pokazao je povećanu peroksidaciju lipida i smanjenu aktivnost antioksidacijskih enzima što može biti zbog izravnoga utjecaja tumora ili zbog drugih čimbenika kao što je tumorska nekroza i/ili upalne promjene. Pojačana rana apoptoza zabilježena je u leukocitima periferne krvi. Primjena vinkristina uz potporno liječenje drugim lijekovima nije dala zadovoljavajuće rezultate zbog uznapredovaloga stupnja zloćudnosti.

Ključne riječi: venerični tumor, metastaze, koža