



Salient features of sticker tumour in dogs and its diagnosis by cytopathology and histopathology technique

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Received: 27 January 2013; Accepted: 10 February 2014

ABSTRACT

Six dogs of various ages, breeds and sex showed tumorous growth confined to extragenital regions. The study was aimed at diagnosing tumorous growth using routine technique clinically and pathologically. Cytological techniques and later the results were compared with routine histopathology. Two male Labrador dogs, 1 female Spitz and 3 male non-descript dogs with tumour masses over and around the genital organ were used. Tumour impression sample and excised tumour were used as material for the study. Fine needle aspiration cytopathology (FNAC) with various cytological stains and routine histopathology with haematoxylin and eosin staining were performed. Grossly, the tumour masses appeared as single or multiple irregular, cauliflower like and had a tendency to bleed and in almost all cases colour was pink to red. Cytologically, the tumour yielded a homogenous, sheet-like high cellular mass. Cytoplasm with punctate vacuoles, anisokaryosis with anisonucleoliosis and coarse to reticulate nuclear chromatin were prominent features. Histopathology showed sheets of round cells with nuclear and cytoplasmic variations. The study concluded that cytopathology could be used as a quick, rapid, field diagnostic technique in combination with histopathology for the diagnosis of TVTs.

Key words: Canine, Cytopathology, Histopathological correlation, Transmissible venereal tumour

Transmissible venereal tumour (TVT), also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or sticker tumour, is an unusual reticulo-endothelial tumour of the dog that mainly affects the external genitalia and occasionally the internal genitalia and adjacent tissues (Amaral *et al.* 2007). Sticker's sarcoma or TVT, a horizontally transmitted neoplasm of the dogs, is transmitted through coitus in both sexes and spreads during routine sniffing or other contact (Fossum 2007). Both males and females were equally affected by plasmacytoid tumors with no definitive breed predisposition (Joao *et al.* 2011). The lesions of TVT are confined to the mucous membranes of the external genitalia and common locations are the genitals, nose, perianal area and it can be localised over skin, brain, oral, nasal and conjunctival mucosa and inguinal lymphnodes (Bostock and Owen 1975, Rogers 1998, Ferreira *et al.* 2000).

It is a locally aggressive tumour with a low tendency for metastatic spread. It is a multi- nodular, invasive, poorly circumscribed tumour usually closely attached to the penis

and prepuce in male dogs and near vulva in female dogs, while its site is dreadful and the accompanying hemorrhagic discharge produces offensive odour. These tumors occasionally metastasize to regional lymphnode, but eventually undergo spontaneous regression after several months (Jones 2006). The management of TVT is not very easy in dogs. It can be done by surgical intervention or radiotherapy or chemotherapy. As it is usually transmitted during coitus, it mainly occurs in young, sexually mature animals (Rogers 1998). Tumour cells of TVT contain an abnormal number of chromosomes ranging from 57 to 64 and averaging 59, in contrast to the normal 78 of the species (Luciano *et al.* 2012). Cytopathology is a very rapid and quick easy diagnostic technique as compared to routine histopathology technique. Hence, the study is designed such that initial diagnosis was made by cytology and later it was compared with histopathology for detection of canine TVTs.

MATERIALS AND METHODS

Six dogs were presented to the clinic, Chandigarh, India, with the history of swelling at prepuce area, blood drooping during and after urination and nodular mass peripheral to extragenitalia. The animals were thoroughly examined, details pertaining to breed, age, site, reproductive factors, and duration of illness were recorded. Fine needle aspiration cytology (FNAC) and also impression smear before surgical excision were taken using 23–25 G needle and 2–5 ml syringe. Surgery was performed aseptically to

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remove the abnormal masses, along with other surrounding structures. Impression smears were made from different areas of the tumour masses. The smears made were air-dried and stained with Wright-Giemsa and Leishman-Giemsa staining. Lesion samples of suspected for tumour were collected in 10% neutral buffer formalin.

The Wright's and May-Grunwald-Giemsa stains were diluted with distilled water in a ratio of 1: 1 and slides were stained for 30 min. The Giemsa working solution was prepared by mixing it with distilled water in the ratio of 1: 10 and stained for 30 min. The Leishman (150 mg) and Giemsa (30 mg) stain was prepared with acetone free methanol (100 mL). The smears were flooded with the stain for 1 min and diluted with double the quantity of distilled water and allowed to stain for 20 min. The smears were evaluated cytologically for initial diagnosis of tumours (Tyler *et al.* 1993).

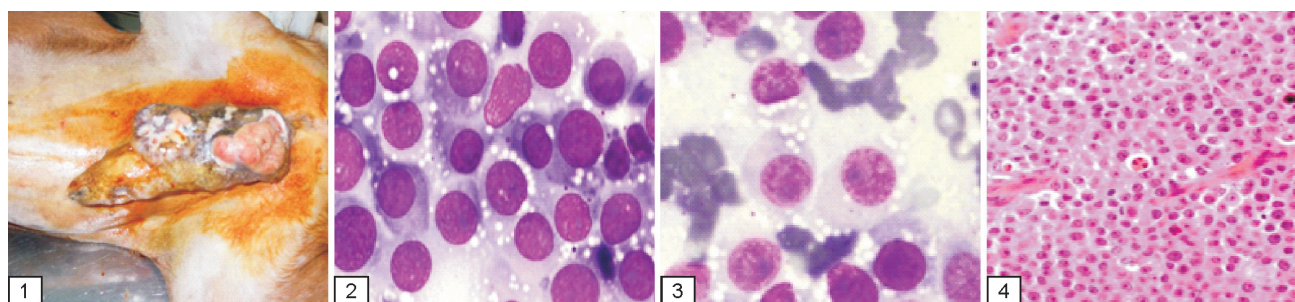
Histology of the tumour: Surgically removed tissue samples were taken from the tumour mass and fixed in 10% neutral buffered formalin. Tissue samples were processed through ascending grades of alcohol, acetone and benzene. Finally tissue samples were embedded in paraffin blocks. Sections of blocks were cut at 3–5 μ m thickness and stained by the haematoxylin and eosin staining. Microscopic evaluation of the lesions and photomicrography of tissue sections was done and finally cellular morphomeric characteristics, cytological features were compared with histopathological features by light microscope.

RESULTS AND DISCUSSION

Grossly, the tumour mass were irregular, cauliflower like, 4–7.5 cm in diameter, reddish at the periphery to prepuccial area and penile area (Fig. 1). The consistency of the mass was soft and friable. Air-dried impression smear revealed cytopathologically prominent presence of cytoplasmic vacuolation (Fig. 2) (Spugnini *et al.* 2008). The size and number of individual punctate vacuoles vary with tumour cell morphology. The nature of cellularity was high with homogenous round individual cells arranged in a sheet-like pattern. A great variation in the cellular (anisocytosis), nuclear morphology (anisokaryosis) and various stages of mitosis (Fig. 3) were observed. The nucleus of the tumour

cells was oval to round nuclei and usually centrally located and had a prominent nucleolus and finely stippled chromatin. Examination revealed that typical round to slightly polyhedral cells; anisonucleoliosis were prominent in the nucleus of the tumour cells. Histopathologically TVT had neoplastic foci which were composed of diffuse sheets of round cells and scant amounts of connective tissue stroma. In some areas, neoplastic cells were arranged in pseudo-alveolar pattern with intervening delicate fibrous stroma (Fig. 4). Individual neoplastic cells had a round hyperchromatic nucleus with coarsely stippled chromatin and a distinct eosinophilic nucleolus. The cytoplasm was moderate and granular with indistinct cytoplasmic borders. The cells were baso-eosinophilic with Haematoxylin and Eosin (H&E) stains. Anisokaryosis was mild to moderate, and mitotic rates were high, ranging from 3 to 7 mitoses per 400 $\times$ microscopic fields. Necrosis and haemorrhage were present in some areas. Sometimes, they grow in rows, cords, or loose in a delicate stroma. The presence of immature lymphocytic round individual cells forming shape of variable size and in an arborizing fibrovascular network.

Tumour masses in various canine species of the size varied from 2.0 cm up to as much as 15 cm in diameter (Shakir and Sundararaj 1994) and they can be multiple nodular masses in the external genitalia noted by Maclachlan and Kennedy (2002) and Purohit (2009). The nuclear chromatin pattern was coarse to reticulate. TVT cells that lack cytoplasmic vacuoles may be easily confused with other round cell tumours. The morphological appearance and location of the tumour however could help in the diagnosis. So, definitive diagnosis could be based on physical examination and cytological findings typical of TVT in exfoliated cells obtained by swabs, fine needle aspiration or imprints of the tumours (Moulton 1978). As the tumour mass increases, the cells become tightly packed and irregular in shape and fibroblasts appear, perhaps an indication of the transformation of tumour cells (Kennedy *et al.* 1977, Calvet *et al.* 1982). There was minimal to mild perivascular infiltration of lymphocytes, plasma cells and rare macrophages. Arrangement of tumour cells ranged from the initial glandular appearance to pallisading and compacted cells (Tella *et al.* 2004).



Figs 1–4. 1. Gross image of TVT over the penis. 2. Microphotograph of impression smear showing scattered round tumor cells showing distinct vacuolation of suggestive of TVT. 3. Microphotograph of impression smear showing scattered round tumor cells suggestive with various stages of mitosis and vacuolation in round cells TVT. 4. Diffused sheets of round cells and scant amounts of connective tissue stroma. Individual neoplastic cells had a round hyperchromatic nucleus with a distinct eosinophilic nucleolus and stippled chromatin. The cytoplasm was moderate and granular, with distinct cytoplasmic borders. Anisokaryosis was mild to moderate. H and E.

This tumour could easily be distinguished from other round cell tumours by a simple algorithm. Mitotic figures in different stages of mitosis were prominent. This indicated the proliferating nature of the tumour cells. Similar cytological features were reported by many workers (Alleman and Bain 2000, Meinkoth and Cowell 2001). These observations concurred with those of earlier studies (Krithiga *et al.* 2005) However, on routine H and E stained slides, the nuclear and cytoplasmic differences between TVT and histologically similar histiocytomas can be subtle. In this study, post-surgical survivability was also analysed. No metastasis was observed, since TVT are immunogenic tumours and the immune system of the host might have played a role in inhibiting tumour growth and metastasis (Cohen 1985). Moreover, reports also indicated less than 5–17% of metastasis in canine species (Richardson 1981).

Typical TVT cells were with a round nucleus and a thin rim of light blue, vacuolated cytoplasm (Kumar *et al.* 2012). Nucleo-cytoplasmic ratio ranged from 1: 1 to 4: 1. Anisokaryosis and anisocytosis were mild to moderate as also noted by Park *et al.* (2006). TVT is one of the most commonly occurring round cell tumours and poses great difficulties in differentiation. It displays histological resemblance to canine cutaneous histiocytomas and other round cell tumours, thereby presenting great difficulties for pathologists in their differentiation (Pawaiya *et al.* 2006).

From the above points, we conclude that transmissible venereal tumour (TVT) is the most prevalent neoplasia of the external genitalia of the dog in tropical and sub-tropical areas. Cytology could be used as a quick, rapid field diagnostic technique in combination with histopathology for the diagnosis of TVT.

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