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Cytological diagnosis and its histological correlation in canine transmissible venereal tumour

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ABSTRACT

The study was aimed at diagnosing transmissible venereal tumour (TVT) initially using cytological techniques and later the results were compared with routine histopathology. Two Spitz and one Labrador bitches with tumour masses of about 4-7.5 cm in diameter seen on the vagina and vulva were used as material for the study. Fine needle aspiration cytology (FNAC) with various cytological stains and routine histopathology with haematoxylin and eosin (H&E) staining were performed. Grossly, the tumour masses appeared irregular, cauliflower like and had a tendency to bleed. 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 cytology could be used as a quick, rapid, field diagnostic technique in combination with histopathology for the diagnosis of TVTs.

Key words: canine, cytology, histopathological correlation, transmissible venereal tumour

Introduction

Transmissible venereal tumour (TVT), also known as infectious sarcoma, venereal granuloma, transmissible lymphosarcoma or sticker tumour is an unusual reticulo-

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endothelial tumour of the dog that mainly affects the external genitalia and occasionally the internal genitalia (GOLDSCHMIDT and HENDRICK, 2002). As it is usually transmitted during coitus, it mainly occurs in young, sexually mature animals (ROGERS, 1997). 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. Though histopathology is routinely used for the diagnosis of TVTs, little emphasis is placed on cytology, which is a very rapid and field diagnostic technique. In the present scenario, cytopathology plays a major role in diagnosing many tumours. 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

Three tissue samples (Two from Spitzes and one from a Labrador) suspected for TVT were collected from bitches, which came for treatment to the Department of Clinics, Veterinary College and Research Institute, Namakkal, Tamilnadu. The animals were thoroughly examined, details pertaining to breed, age, site, reproductive factors, duration of illness and wherever possible particulars of spaying, number of pregnancies and periodicity of oestrus were gathered.

Fine needle aspiration cytology (FNAC) before surgical resection, was taken using 23-25 G needle and 2-5 mL syringe. Surgery was then performed to remove the masses, along with other surrounding structures. Impression smears were made from different areas of the tumour masses. The smears made were either wet fixed with 95 per cent ethanol (ALLEN et al., 1986) or absolute isopropanol for 20 min or air dried immediately. The smears subjected for wet fixation were stained with Harris Haematoxylin and Eosin (BANCROFT and STEVENS, 1996), whereas the air dried smears were stained with Wright's (W), Wright-Giemsa (WG), May-Grünwald-Giemsa (MGG) and Leishman-Giemsa (LG) staining techniques.

The Wright's and May-Grünwald-Giemsa stains were diluted with distilled water in a ratio of 1:1 and the 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 then 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 (ALLEN et al., 1986; TYLER et al., 1993).

Representative tissue pieces were also collected from the tumour mass and were fixed in 10 per cent buffered formalin, processed through alcohol and xylol and embedded in paraffin. Sections were cut at 3-5 m thickness and stained by the Haematoxylin and Eosin

staining technique. Finally cytological features were compared with histopathological features.

Results

Grossly, the tumour masses were irregular, cauliflower like, 4-7.5 cm in diameter, reddish in colour and were seen on the vagina and vulva. The consistency of the mass was soft and had a tendency to bleed (Fig. 1 and 2). Cytological examination revealed typical round to slightly polyhedral cells (Fig. 3). The cytoplasm of the tumour cells was grayish in colour when stained with Romanowsky in combination with Giemsa stains. However, the cells were baso-eosinophilic with haematoxylin and eosin (H&E) stains. The nature of cellularity was high with homogenous round individual cells arranged in a sheet-like pattern (Fig. 4). A great variation in the cellular (anisocytosis) and nuclear morphology (anisokaryosis) was observed. The nucleus of the tumour cells was round to oval in shape and centrally placed. Anisonucleoliosis were prominent in the nucleus of the tumour cells. The nucleoli were basophilic and the number varied from one to three. The nuclear chromatin pattern was coarse to reticulate (Fig. 5). The most prominent cytological feature of TVT is the presence of distinct, clear, cytoplasmic vacuoles often referred to as punctate vacuoles with a delineated outline (Fig. 6). The nuclear to cytoplasmic ratio of tumour cells was large. Mitotic figures in different stages of mitosis were prominent (Fig. 7).

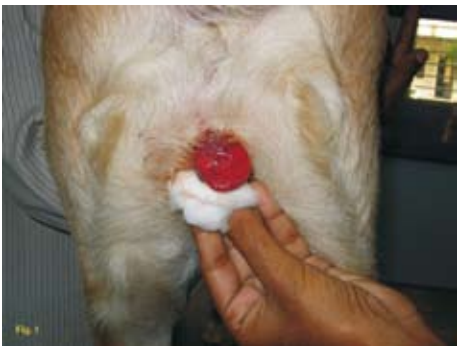


Fig. 1. Spitz dog, transmissible venereal tumour (TVT). Note irrequlars, cauliflower like reddish tumour mass on the vagina.

Fig. 2. Labrador dog, transmissible venereal tumour (TVT). Note irrequlars, cauliflower like reddish tumour mass on the vagina.

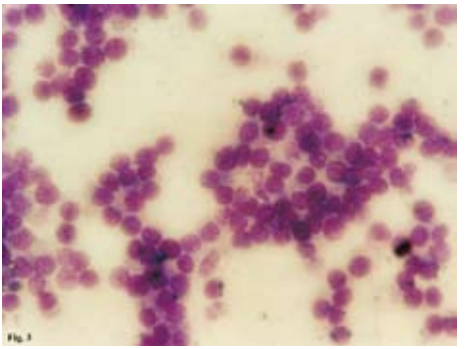


Fig. 3. TVT cytology. Typical round to polyhedral tumour cells. Leishman-Giemsa stain; ×400.

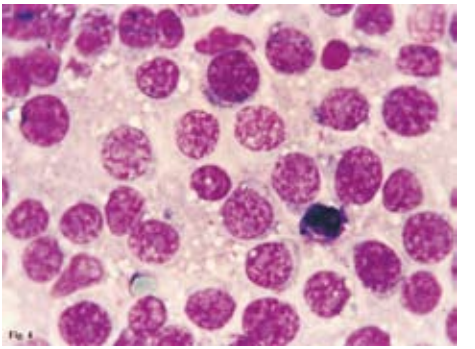


Fig. 4. TVT cytology. Highly cellular, homogenous, round sheet like, individually arranged tumour cells. Leishman stain; ×1000.

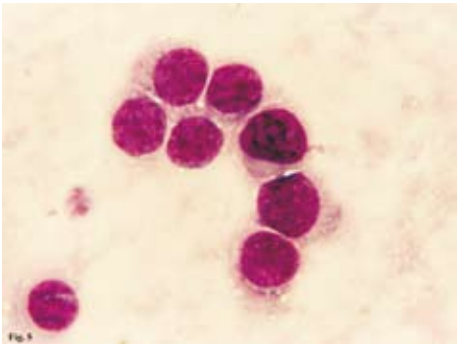


Fig. 5. TVT cytology. Sheet of tumour cells with coarse to reticulate chromatin and basophilic cytoplasm. Leishman-Giemsa stain; ×1000.

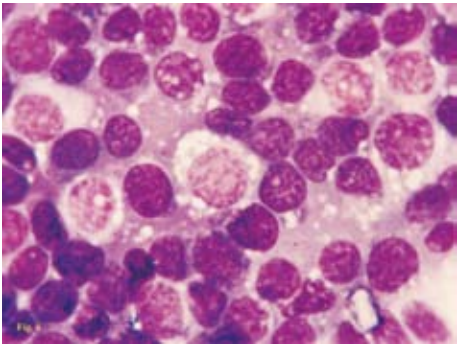


Fig. 6. TVT cytology. Cytoplasm with punctate vacuoles, hyperchromatism and multiple nucleoli. Leishman-Giemsa stain; ×1000.

Histopathology revealed a sheet of round individual cells containing round vesicular nuclei, the borders of which could not be easily differentiated (RICHARDSON, 1981; ROGERS, 1997). The cells were situated in an arborizing fibrovascular network. A distinct single, centrally placed nucleolus with dispersed chromatin was noticed. Stroma was scant. There was frequently an infiltration of lymphocytes, plasma cells and few macrophages. Mitotic figures were also seen (Fig. 8).

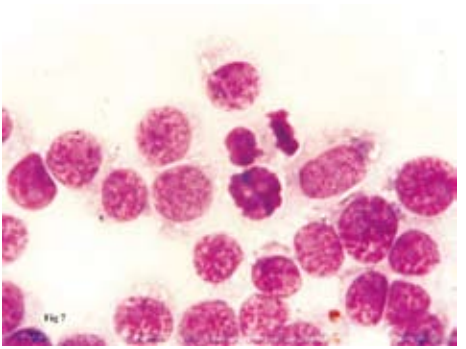


Fig. 7. TVT cytology. Tumour cells with varying stage of mitosis. MGG; ×1000.

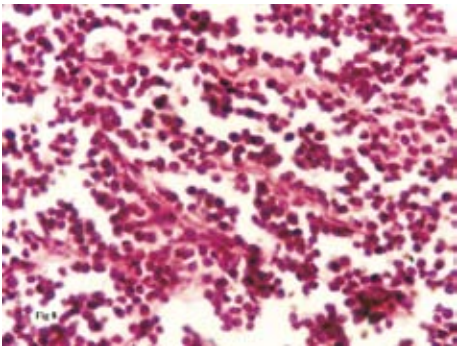


Fig. 8. TVT. Section showing sheet of round cells with vesicular nuclei, arborizing fibrovascular network and scanty stroma. H&E; ×1000.

Discussion

Transmissible venereal tumour is one of the most commonly occurring round cell tumours and poses great difficulties in differentiation because this tumour may have similarities with its close entity, such as a mast cell tumour, etc. The multiple nodular masses in the external genitalia observed in this study were in accordance with earlier observations (GOLDSCHMIDT and HENDRICK, 2003; MACLACHLAN and KENNEDY, 2002; SHAKIR and SUNDARARAJ, 1994). They also observed similar tumour masses in various canine species and the size varied from 2.0 cm up to as much as 15 cm in diameter. The prominent cytological feature of TVT is the presence of cytoplasmic vacuolation. The size and number of individual punctate vacuoles vary with tumour cell morphology. 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.

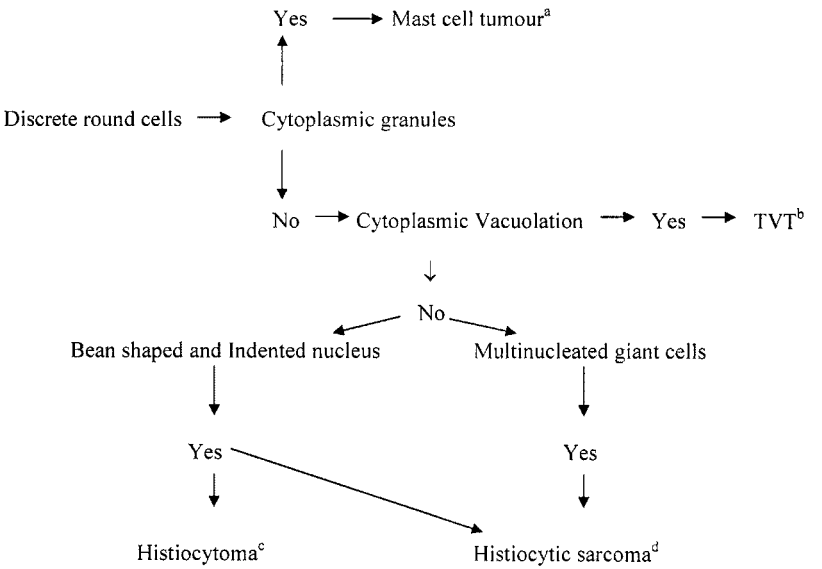
Mitotic figures in different stages of mitosis were prominent. This indicated the proliferating nature of the tumour cells. Similar cytological features were reported by many workers (ALLEMAN and BAIN, 2000; DUNCAN and PRASSE, 1979; FAN et al., 2001; MEINKOTH and COWELL, 2001).

The presence of round individual cells in an arborizing fibrovascular network could help in diagnosing the tumour histologically. These observations concurred with those of earlier studies (KRITHIGA et al., 2005; MACLACHLAN and KENNEDY, 2002). However, on routine H&E stained slides, the nuclear and cytoplasmic differences between TVT and histologically similar histiocytomas can be subtle. So, cytological preparations have better nuclear preservation and should be used to confirm the diagnosis (GOLDSCHMIDT and HENDRICK, 2003).

In the study, post-surgical survivability was also analysed. Even after six months and then one year, 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, 1973; COHEN, 1985). Moreover, reports also indicated less than 5-17 per cent of metastasis in canine species (RICHARDSON, 1981).

Further, TVT 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). 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). This tumour could easily be distinguished from other round cell tumours by a simple algorithm.

An algorithm used in evaluating and differentiating TVTs from other round cell tumours



a. Round cells with eccentric nuclei + Cytoplasmic granulation; b. Round cells with centrally placed nuclei + Cytoplasmic vacuolation; c. Round cells without cytoplasmic granulation or vacuolation + Bean shaped nucleus; d. Round cells without cytoplasmic granulation or vacuolation + Multinucleated giant cells.

Recent approaches are focussed mainly on molecular markers of differentiation using markers like proliferating cell nuclear antigen (PCNA), AgNOR, etc. A recent study attempted to evaluate the usefulness of AgNOR counts, mitotic index and PCNA

index for differential diagnosis of those histologically identical tumours *viz.,* TVT and histiocytomas (PAWAIYA et al., 2006). Studies also indicated the differences in cell types for the progression of TVT. A tumour in the progressive growth stage usually has round cells with microvilli while a regressing tumour presents transitional, rather fusiform cells. Moreover regressing tumours have a high number of T-lymphocytes. (YANG et al., 1976; HILL et al., 1984)

Conclusion

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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SAŽETAK

Istraživanje je provedeno radi uvođenja citološke metode za dijagnosticiranje prenosivoga veneričnoga tumora u pasa. Citološki nalazi uspoređeni su s patohistološkim nalazima. U istraživanje su uzete dvije kuje pasmine špic i jedna pasmine labrador s tumorima stidnice i rodnice promjera od 4 do 7,5 cm. Nakon aspiracije tankom iglom proveden je citološki postupak bojenja različitim bojama te uobičajeno patohistološko bojenje hematoksilinom i eozinom. Tumori su patomorfološki bili nepravilne građe poput cvjetače sa sklonošću krvarenju. Citološki su se doimali homogeno, s gustim staničnim nakupinama posloženim u pločastom obrascu. Ustanovljene su točkaste vakuole u citoplazmi, zatim anizokarioza s anizonukleoliozom i grubim nakupinama jezgrenoga kromatina. Patohistološki ustanovljene su okrugle stanice s promjenama u jezgri i citoplazmi. Citološka metoda može se rabiti kao brzi terenski dijagnostički postupak u kombinaciji s patohistološkim nalazom.

Ključne riječi: pas, citologija, patohistologija, prenosivi venerični tumor