



Equine Sarcoid-histomorphological, Histochemical and Immunohistochemical Studies in A Thoroughbred Horse

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ABSTRACT

Background: Equine sarcoid is a rare equine skin tumour seen in any age group of horses, usually in younger horses. Grossly, it appears as multinodular masses which may or may not ulcerate and pink to greyish white coloured. It was suggested that the papilloma virus is a causative organism along with predisposing factors like skin abrasions and wounds. The definitive diagnosis is based on histopathology and classified according to their gross appearance and clinical behavior. To find out the origin and proliferative nature, histochemical and immunohistochemical study was performed. The present communication reflects various patterns of sarcoid on histopathological examination and it was confirmed by histochemistry and immunohistochemistry.

Methods: A four-year-old bay colt was presented to the Madras Veterinary College Teaching Hospital with nodular masses on the buccal cavity. Fine needle aspiration biopsy (FNAB), blood, serum and tumour biopsy materials were collected under local anesthesia. The collected samples were subjected to Leishman-Giemsa staining, haematological and biochemical analysis, histopathology, histochemical and immunohistochemical examination.

Result: Histopathologically, there was mucosal hyperplasia and hyperkeratosis. The neoplastic cells were arranged as storiform or whorl-like pattern. Also, there was perpendicular orientation of fibroblasts towards the basement membrane (picket fence) at the dermo-epidermal junction, which was considered as characteristic feature of sarcoid. Histochemical examination with Picrosirius red revealed strong positivity characterised by deep red coloured mature collagen fibres. Immunohistochemically, the hyperplastic epithelial cells were positive for pan cytokeratin and fibroblast cells were strongly positive for vimentin. Based on the histopathology, histochemistry and immunohistochemistry, the condition was diagnosed as sarcoid which is rare in horses.

Key words: Equine sarcoid, Histochemistry, Histopathology, Immunohistochemistry.

INTRODUCTION

Equine sarcoid, which is a rare skin tumour in horses and it occurs all over the body but sites of predilection include paragenital region, thorax, abdomen and head and frequently occur at sites of previous injury and scarring (Knottenbelt *et al.*, 2005). Grossly, it appears as elevated, fleshy, multinodular masses which may be ulcerated and appear as pink or greyish white on sections (Meuton, 2017). It is widely accepted that Bovine papilloma virus infection is necessary for sarcoid development but, infection alone is not sufficient for tumor production (Bogaert *et al.*, 2011). It seems virus may have gained entry through the skin abrasions or wounds and resulted in neoplastic transformation of fibroblasts. Sarcoid are classified according to their gross appearance and clinical behaviour. They are classified into six distinct types comprises of nodular, occult, verrucous, fibroblastic, mixed and malevolent (Martens *et al.*, 2000). A definitive diagnosis of sarcoid requires histopathology but biopsy-induced trauma or irritation may exacerbate the lesion and induce proliferation (Knottenbelt *et al.*, 2005). It does not metastasize but can produce severe impact on the function of organs. Large growth in preputial cavity may prevent penile protrusion and compression of urethra may also occur. The present paper represents the various pattern of sarcoid on histopathology and it was confirmed by histochemistry and immunohistochemistry.

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MATERIALS AND METHODS

A four-year-old bay colt was presented to the Madras Veterinary College Teaching Hospital with nodular masses on the buccal cavity. FNAB was performed from the mass as per the standard procedure. Smear was prepared and stained with Leishman-Giemsa stain as described by (Garbyl *et al.*, 2006). Hemogram and biochemical estimations were performed by using automated cell counter and automated biochemical analyser with standard diagnostic kits. Tumours biopsy was taken under local anesthesia and sent to the Department of Veterinary Pathology for confirmatory diagnosis. The tissue samples were fixed in 10% formalin

and embedded in paraffin wax. Sections of 5 µm thickness were prepared and stained with Hematoxylin and Eosin and Picosirius red for histopathological and histochemistry analysis, respectively. Immunohistochemical staining of pan cytokeratin and vimentin were performed as per recommendation of the manufacturer (Bio genex, USA) using super sensitive labelled poly horse radish peroxidase (HRP) polymer method.

RESULTS AND DISCUSSION

Hematological and biochemical values were within the normal range. Cytological examination of stained smear revealed the few clusters of keratinized epithelial cells. Histologically, equine sarcoid is characterized by the presence of proliferating fibroblast cells arranged in whorls, interlacing bundles or haphazard arrays in the dermis and a distinct "picket fence appearance" at the epithelial interface. Epidermal thickening with hyperkeratosis, parakeratosis and rete pegs can also be seen. Occasionally, intranuclear inclusion bodies may be observed (Hallamaa *et al.*, 2005). Histopathological examination revealed the presence of loosely arranged fibrovascular stroma. Few lymphocytes were also noticed. Overlying epidermis showed hyperplasia, orthokeratotic hyperkeratosis and variably sized keratohyaline granules clumped within the keratinocytes. The dermis part consists of spindle shaped fibroblasts cells with variably sized proliferating collagenous tissue. Finger like projections or rete pegs (Fig 1) were found to be extending in to the fibromatous tissue. The neoplastic cells were arranged as storiform or whorl-like pattern (Fig 3). There was also perpendicular orientation of fibroblasts towards the basement membrane "picket fence" (Fig 2) at the dermo-epidermal junction and neovascularisation (Fig 4) which was considered as characteristic feature of sarcoid. The histopathological findings recorded in this case were similar with the findings of earlier workers (Goodrich *et al.*, 1998). Equine sarcoid are frequently encountered in young horses mostly between 3 to 6 years of age, mostly in head, neck and limbs. However, in this case, it was noticed in four years and tumour was present in the buccal region. Sarcoid is considered as tumour of fibrous tissue although epidermal involvement is a feature in most cases, but it is not obligatory. In this case, more fibroblast involvement was present. Picosirius red staining is a useful staining technique applied for study of collagen pattern in normal and pathological tissues (Lattouf *et al.*, 2004). Strong positive staining is indicated by deep red coloured mature collagen fibers in the dermis (Fig 5), as compared to pink coloured immature collagen fibers, was observed in the present communication. This staining can be used for confirmatory diagnosis of collagen invasion into the epithelium of sarcoid. Immunohistochemistry is one of the additional tools for finding out the origin and tumour proliferation. Immunostaining of pan cytokeratin is positive for epidermal cells, which was strongly positive in proliferative epidermal cells. Similar observations were recorded by the earlier



Fig 1: Sarcoid-Epidermal hyperplasia, hyperkeratosis and Rete peg - H&E - Bar = 4 µm.

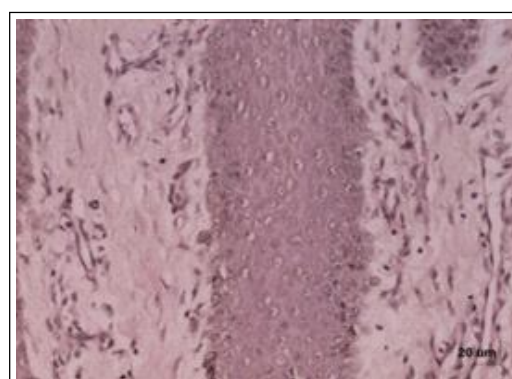


Fig 2: Sarcoid-Picket fence and fibroblast perpendicular - H&E - Bar = 20 µm.

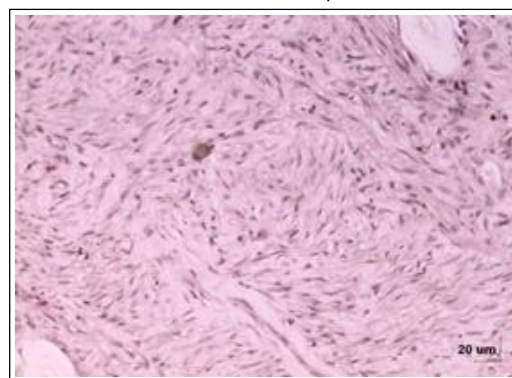


Fig 3: Sarcoid-Whorl pattern - H&E - Bar = 20 µm.

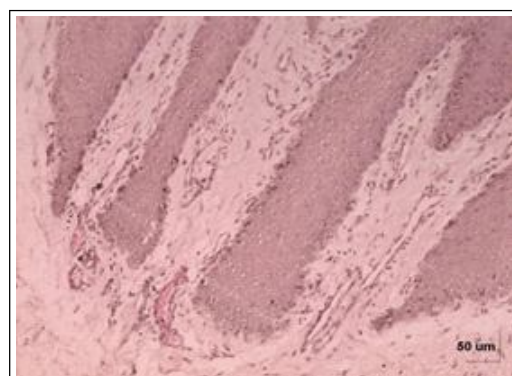


Fig 4: Sarcoid-Neovascularization - H&E - Bar = 10 µm.

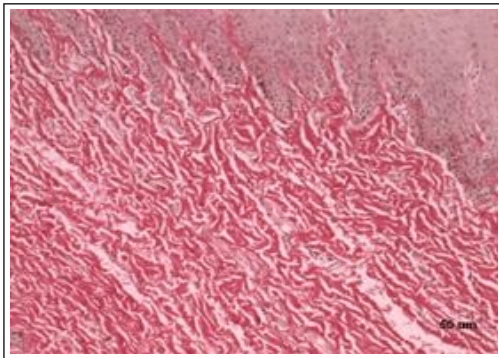


Fig 5: Sarcoid-Mature collagen-Picosirius red-Bar = 10 µm.

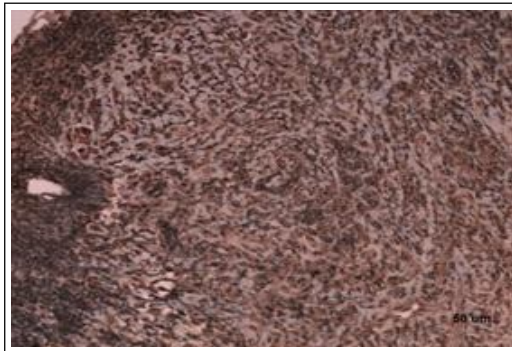


Fig 6: Sarcoid-Mature collagen-Vimentin-Strong positive-Immunohistochemistry-Bar = 10 µm.

workers (Kasperowicz *et al.*, 2006). Immunohistochemistry for vimentin is strongly recommended for identification of mesodermal origin of tumours. Sarcoid is characterized by an increased number of mature collagen fibers. Fibroblast in sarcoid express vimentin indicating its mesodermal origin (Bogaert *et al.*, 2011). Strong positivity of vimentin was observed in this case, which appeared as finely granular in the cytoplasm of fibroblast and myofibroblasts (Fig 6). It seems that the histopathological description, histochemical and immunohistochemical expression of pan cytokeratin and vimentin have greater importance in the treatment and prognosis of sarcoid.

SUMMARY

A case of sarcoid was diagnosed and recorded in a four-year-old thorough breed horse. Classification, histopathological, histochemical and immunohistochemical changes were studied and reported.

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