PATHO-MORPHOLOGICAL AND AGNOR BASED DIAGNOSIS OF CUTANEOUS SQUAMOUS CELL CARCINOMA IN BUFFALO

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ABSTRACT

A case of cutaneous squamous cell carcinoma was reported in a 4 year old buffalo. Grossly, growth mass was 10 cm long, 7 cm width and 175 gm in weight and located on cranial aspect of left hind limb at fetlock joint. Histologically, the tumour showed irregular mass or cords of epidermal cells that proliferate downward and invade the dermis and sub-cutis. Keratin pearl formation also occurs at some places and it was composed of concentric layers of squamous cell gradually increasing keratinisation towards the centre. The value of mAgNOR and pAgNOR count for cutaneous squamous cell carcinoma was found to be 2.72±1.05 and 30%, respectively. On the basis of gross, histological examination and AgNOR count, the tumour was led to the final diagnosis of squamous cell carcinoma.

Keywords: *Bubalus bubalis*, buffaloes, mAgNOR, pAgNOR, cutaneous squamous cell carcinoma, Keratin pearl

INTRODUCTION

Squamous cell carcinoma is a malignant tumour of squamous epithelial cells. It is a common neoplasm affecting all domestic animals. There is no sex predisposition for the squamous cell carcinoma of the skin, and this tumour is found in all areas of skin of domestic animals. During the development of tumour, firstly there is a loss of hair, scaling, crust formation and ulceration occur finally (Moulton, 1990). Kohli *et al.* (2008); Devi *et al.* (2010) reported squamous cell carcinoma in the ear and at the dorsal area of neck, respectively in buffaloes. The Argyrophilic nucleolar organizer regions (AgNOR) are non-histone proteins which are associated with ribosomal genes (rDNA) localized in the nucleolar organizer region (NOR) (Crocker *et al*., 1989). AgNOR is a molecular marker used to study the rapidity of cell proliferation in various types of tumors as well as to aid in diagnosis and prognosis (Kumar *et al*., 2010). There is sufficient literature on occurrence of squamous cell carcinoma in cattle, but the occurrence of said tumour has been sparsely reported in buffaloes.

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MATERIALS AND METHODS

A female buffalo of 4 years old was presented for treatment in the Department of Teaching Veterinary Clinical Complex, Faculty of Veterinary Sciences and Animal Husbandry, R.S Pura, Jammu, India with a history of injury since 2 months back, healed but after that lead to the growth on cranial aspect of left hind limb at fetlock joint. The animal was healthy and had normal feed intake. The tumour mass was taken after surgery and fixed in 10% neutral buffered formalin and processed by routine paraffin embedding method. After 3 to 4 days of fixation, the tissues were washed in running water for 7 to 8 h, dehydrated in ascending grades of ethyl alcohol, cleared in benzene and embedded with melted paraffin wax (melting point 58°C). The paraffin blocks were prepared and the sections were cut at 4 to 5 µ thickness with a hand operated microtome. The paraffin embedded sections were then passed through sequential steps of deparaffinisation in xylene, rehydration by passing through descending grades of ethyl alcohol to running tap water and stained by routine haematoxylin and eosin stain. The H and E stained slides were observed under microscope and lesions were recorded (Bancroft and Gamble, 2002). AgNOR study was conducted on the specimen collected for histopathology. The microtome cut tissue section was stained for AgNOR by using the method described by Crocker *et al.* (1989) with some modifications. For this, formalin fixed 4 to 5 µ paraffin sections were stained with a solution made up of 50% silver nitrate solution (prepared fresh) and 2% gelatine stored at room temperature. Working solution was freshly prepared using two parts of silver nitrate solution and one part of gelatine solution. To carry out AgNOR staining, the cut sections were deparaffinised and rehydrated using distilled de-ionized water. Then place in plastic jar filled with the staining solution and incubated at 37°C in dark for 60 minutes and washed thoroughly with distilled deionised water and dehydrated. After drying, the slides were cleared in xylene, mounted in DPX mountant (Kumar *et al*., 2010) and visualized under microscope under 100 x using oil immersions. The AgNORs were visualized as brown-black discrete dots of variable size within the nuclei. AgNOR in 100 cells was counted and size variation was also recorded. Two counts were performed. The first count was the mean number of AgNORs in 100 tumour nuclei (mAgNOR). The second count was the percentage of nuclei exhibiting five or more AgNOR granules/nucleus/100 cells called proliferative index (pAgNOR).

RESULTS AND DISCUSSION

On gross examination, the tumour mass was hard, whitish in colour with 10 cm long, 7 cm width and 175 gm in weight and located on cranial aspect of left hind limb at fetlock joint (Figure 1). Mass was excised surgically under local anaesthesia. Histopathological examination showed irregular mass or cords of epidermal cells which proliferated downward and invaded the dermis and sub-cutis (Figure 2). Keratin pearl formation was also observed. These cancer pearls were composed of concentric layers of squamous cell showing gradually increasing keratinisation towards the centre (Figure 3). Mitotic figures were also visualized. The gross and the histopathological findings were similarly reported by Moulton (1990); Jones *et al*. (1997); Kohli *et al*. (2007). Devi *et al.* (2010) reported islands or cords of neoplastic

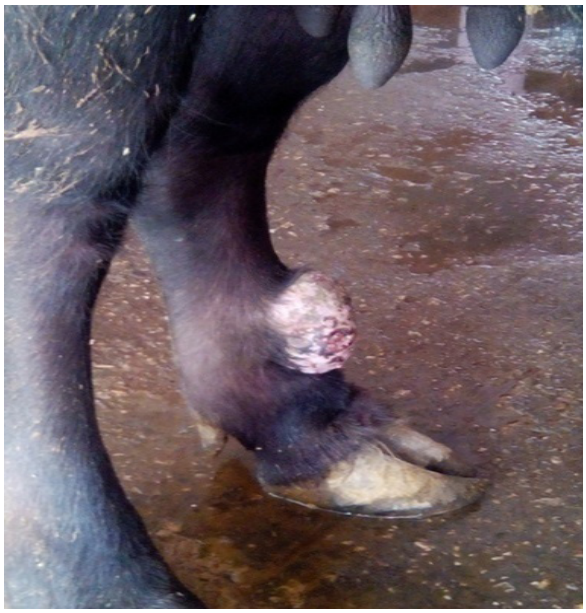

Figure 1. Cutaneous squamous cell carcinoma showing hard protruded whitish colour, ulcerative mass on cranial aspect of left hind limb at fetlock joint which bled very easily.

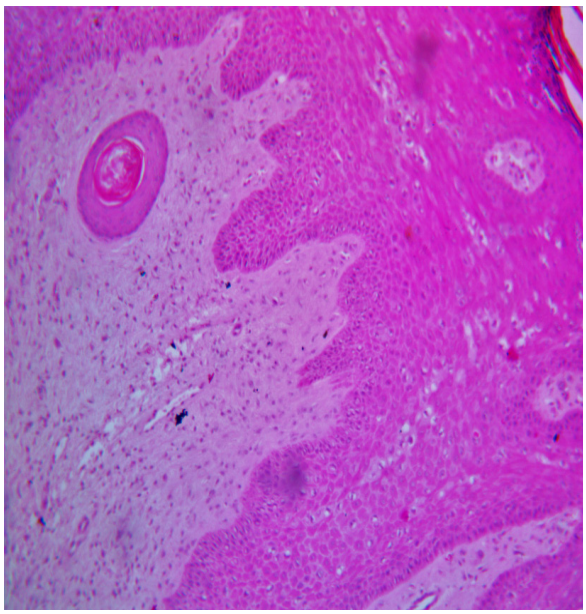

Figure 2. Photomicrograph of cutaneous squamous cell carcinoma showing irregular mass or cords of epidermal cells that proliferate downward and invade the dermis and sub-cutis. H&E X100.

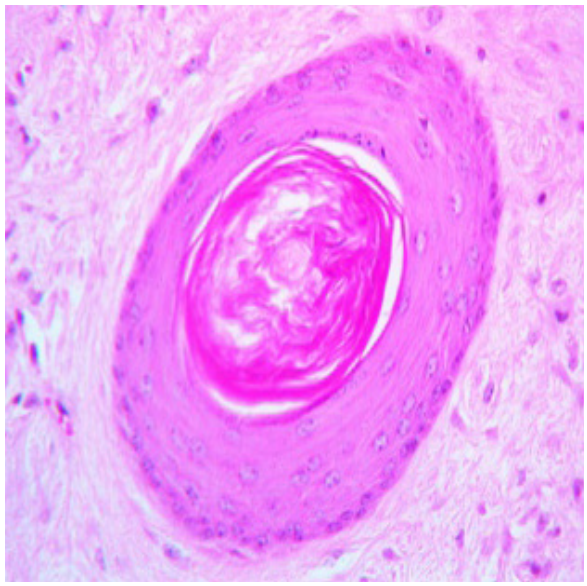

Figure 3. Keratin pearl composed of concentric layers of squamous cell gradually increasing keratinisation towards the centre. H&E X400.

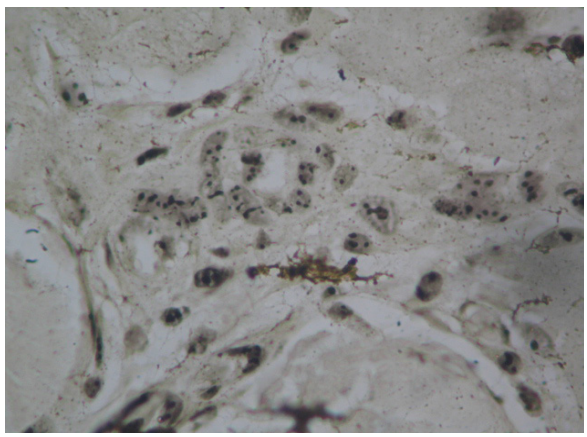

Figure 4. Cutaneous squamous cell carcinoma showing numerous round to irregular, dark brown to black AgNOR dots of varying sizes. AgNOR X400.

squamous epithelium with epithelial pearls at some places in growth in Indian buffalo. Baniadam *et al*. (2010) reported firm, white dermal masses characterised by keratinisation, vesicular nuclei with prominent nucleoli.

AgNOR staining showed presence of black dots within the nucleus of the neoplastic cells (Figure 4). The value of mAgNOR and pAgNOR count for cutaneous squamous cell carcinoma was found to be 2.72±1.05 and 30%, respectively. These findings were in accordance with the findings made by Crocker *et al*. (1989); Chandravathi *et al*. (2013); Veena *et al*. (2014). Chandravathi *et al*. (2013) reported 40 samples of epithelial tumours in canine in which one case of squamous cell carcinoma showed mAgNOR to be 6.23±1.42. Gulia *et al*. (2011) also reported squamous cell carcinoma in the oral cavity in human with the value of mAgNOR of three grades of oral squamous cell carcinoma was 4.71±0.81, 6.34±0.89 and 8.70±0.65. AgNOR dots were small, homogenously stained in benign tumour and as the grade of tumour rises the AgNOR dots became irregular and large. Agarwal *et al.* (2014) reported AgNOR as a reliable indicator of cell proliferation and allowed a clear distinction between benign, premalignant and malignant epithelial changes. Malignant tumours had numerous, smaller and irregular AgNORs, dispersed throughout the nucleus whereas benign tumours had large, round, sharply defined and few AgNOR dots confined to the nucleoli (Chandrasekar and Lalitha, 1995).

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