Haryana Vet. (March, 2020) **59(SI),** 31-33

Research Article

IMMUNOHISTOCHEMICAL EXPRESSION OF CYTOKERATIN 14 AND p53 IN BOVINE ORAL TUMOURS

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Received: 11.04.2019; Accepted: 08.07.2019

ABSTRACT

Oral tumours in production animals are generally rare, only sporadic occurrence is observed. These neoplasms cause potentially serious problems in feeding and mastication. The present study was conducted on the tumourous growths collected from 29 clinical cases of cattle and buffaloes bearing oral growths. Present study was carried out with an aim to evaluate the immunohistochemical expression of cytokeratin (CK) 14 and p53 in bovine oral tumours. Histologically, these were classified as epithelial tumours such as ameloblastoma (9), papilloma (1), adenoma (1); mesenchymal tumours such as fibroma (7), myxoma (3) and mixed type tumours (8). All epithelial tumours revealed intracytoplasmic expression of CK14; however, no immunoreactivity was noticed in mesenchymal tumours. None of the tumours showed immunohistochemical expression of p53. From the present findings, it is concluded that CK expression in epithelial tumours differentiated these from mesenchymal tumours. No expression of p53 indicated that the p53 mutants may not play an important role in bovine oral tumours.

Keywords: Cytokeratin 14, Immunohistochemistry, Oral tumours, p53

Oral tumours in production animals are generally rare however, only sporadic occurrence is observed (Meuten, 2017). Oral tumours are observed as mass-like lesions causing serious problems in feeding and interfering with mastication in young cattle (Head *et al.*, 2002). Histopathology is the gold standard test for diagnosis of tumours. However, immunohistochemistry (IHC) using tumour marker plays an important role in diagnosis of tumours, its origin and to ascertain the possible role of oncogenes in carcinogenesis (Tanno *et al.*, 2006). Although, a considerable research work has been carried out on human tumours associated antigens; however, there is limited research work has been done in tumours of cattle and buffaloes. Cytokeratin (CK) is one of the most important tumour markers used for differentiation of epithelial tumours and belongs to the intermediate filament (IF) protein family. At present, more than 20 different CKs have been identified, of which CKs 7, 8, 10, 14, 18, 19 and 20 are the most abundant in simple epithelial cells (Barak *et al.*, 2004). Expression of CK14 and CK19 serve as indicators for malignant transformation in oral carcinogenesis (Yoshida *et al.*, 2015). p53 is a tumour suppressor gene which prevents the growth of aberrant cells by cell cycle arrest, DNA repair or apoptosis. It is a significant marker of carcinogenesis and can be considered as an important marker for clinical evaluation, diagnosis and prognosis (Ghanghoria *et al*., 2015). Keeping above facts in view, present study was carried out with an aim to evaluate the immunohistochemical expression of CK 14 and p53 and its role in bovine oral tumours.

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MATERIALS AND METHODS

Present study was conducted on the tissue samples collected from 29 clinical cases of cattle and buffaloes bearing oral growths presented to Department of Veterinary Clinical Complex, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. The representative tissue samples were collected and fixed in 10% buffered formalin.

After proper fixation, tissues were processed for paraffin embedding technique. The paraffin embedded tissues were cut into 4µm thick sections using semi-automatic microtome and sections were mounted on APES coated slides. Immunohistochemistry was carried out as per the method described by Sharma *et al.* (2015). For CK 14, intra-cytoplasmic brick red or brown red staining was considered as positive; while, for p53, brick red colour in nuclei of neoplastic cells were taken as positive.

RESULTS AND DISCUSSION

Immunopositive reactivity for CK 14 was observed in epithelial tumours. However, no CK-14 immunoreactivity was noticed in mesenchymal tumours. Epithelial origin viz. ameloblastoma, fibropapilloma (Fig. 2), fibromatous epulis with adenoma and hyperplasia of epithelial cells showed positive immunoreactivity for CK 14 and negative immunoreactivity for myxoma, fibroma and chondroma of mesenchymal origin. These epithelial tumours revealed intra-cytoplasmic brick red or brown red immunoreactivity. These results support the fact that cytokeratins are considered as tumour markers of epithelial origin as during transformation of normal epithelial cells into neoplastic cells, cytokeratin patterns

are usually maintained (Chu and Weiss, 2002).

The intensity of cytoplasmic reactivity of CK14 was intense in tall columnar cells while reticular mesenchymal cells showed on immunoreactivity in ameloblastoma (Fig.1). These finding co-relate with Chavan *et al.* (2014) who revealed that ameloblastoma in eight year old bullock diagnosed histologically, was immunohistochemically positive for CK8, CK18 and vimentin. No immunoreactivity for CK14 in odontogenic myxoma is seen. These findings are in correspondence with WHO classification. Immunohistochemistry of odontogenic myxoma in present case were in accordance with that reported by earlier workers in odontogenic myxoma of the mandible in filly (Chandra *et al.*, 1999) and dog (Barigye *et al.*, 2011). Cytoplasmic immunostaining for CK 14 in epithelial cells of fibropapilloma was positive while fibrous tissue showed no immunoreactivity (Fig.2). Cytoplasmic immunoreactivity for CK 14 in hyperplastic epithelial cells of epulis and myoepithelial cells of glands in adenoma was found positive (Fig. 3). Cytokeratins (CK) are abundant in keratinized cells, particularly CK14 and CK19, which are expressed in stratified squamous epithelial cells (Yoshida *et al.*, 2015).

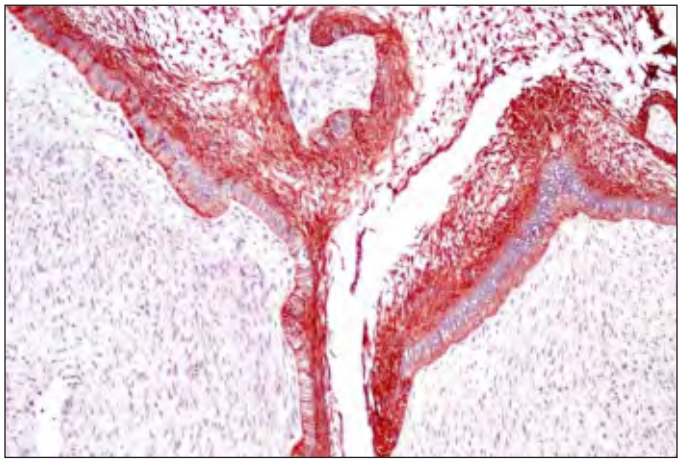

Fig. 1. Photomicrograph of ameloblastoma showing intense red coloured cytoplasmic immunostaining for CK 14 in epithelial cells and no immunoreactivity in mesenchymal cells. IHC × 100

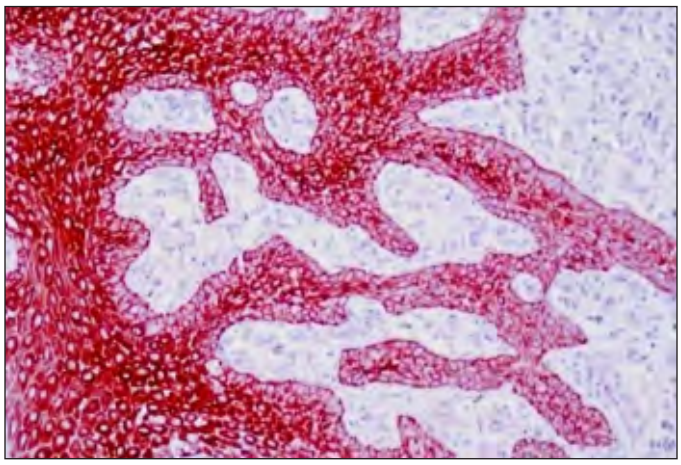

Fig. 2. Photomicrograph showing cytoplasmic immunostaining for CK 14 in epithelial cells of fibropapilloma. IHC × 200

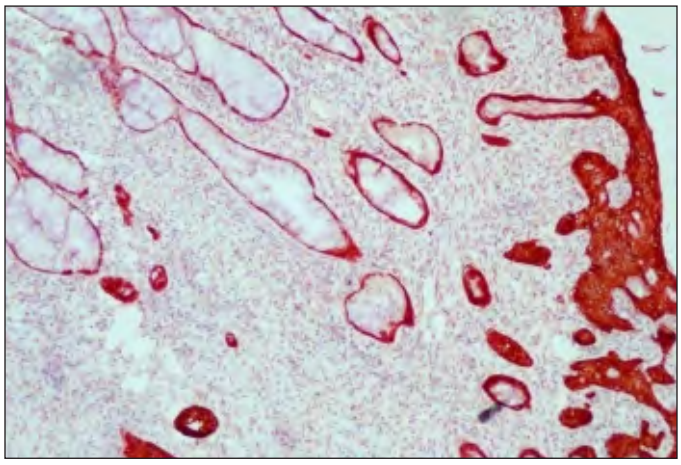

Fig. 3. Photomicrograph showing cytoplasmic immunoreactivity for CK 14 in hyperplastic epithelial cells of epulis and myoepithelial cells of glands in adenoma. IHC × 100

In the present study, no bovine oral tumour was

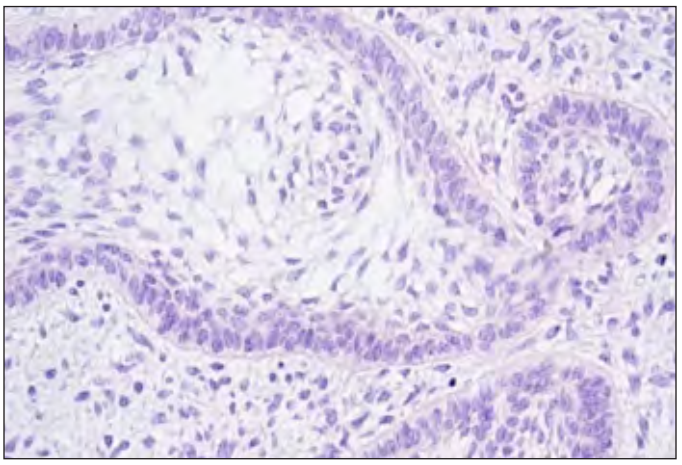

Fig. 4. Photomicrograph of ameloblastoma showing no immunostaining for p53. IHC × 200

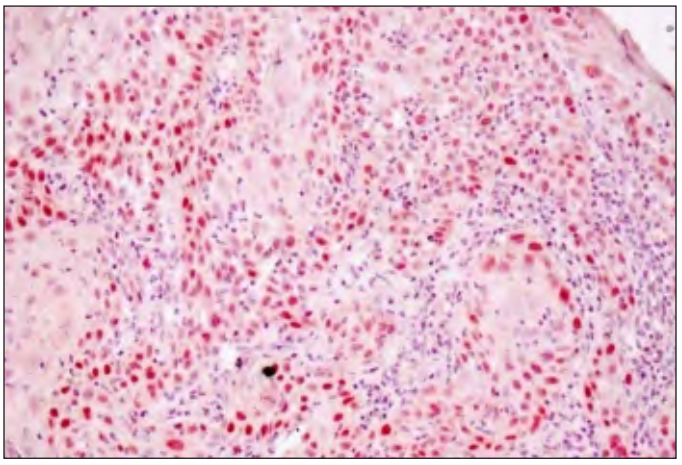

Fig. 5. Photomicrograph showing moderate to intense nuclear p53 immunostaining in neoplastic cells of bovine ocular squamous cell carcinoma (Positive control). IHC × 200

found positive for p53 immunoreactivity (Fig. 4) may be because of all the bovine oral tumours were benign in nature and p53 immunoreactivity is more common in malignant tumour. p53 was found positive in ocular squamous cell carcinoma in cattle (Fig. 5) taken as positive control with the same anti p53 antibodies. It indicated that p53 mutants may not play role in the oncogenesis of bovine oral tumours. Similar finding was also observed by Sharma (2015) who did not find p53 activity in odontogenic myxoma and ameloblastoma in bovine; however, the immunohistochemical expression of p53 was reported in odontogenic myxoma in jaws of human beings (Iezzi *et al.*, 2007). p53 over expression was frequent in bovine ocular squamous cell carcinoma and no correlation between the percentage of p53 stained nuclei and the degree of differentiation was observed (Carvalho *et al.*, 2005).

From the present study, it is concluded that cytokeratin expression in epithelial origin tumours differentiated these from mesenchymal tumours. No expression of p53 indicated that the p53 mutants may not play important role in bovine oral tumours.

ACKNOWLEDGEMENT

Author is highly thankful to Indian Council of Medical Research (ICMR) for providing Junior Research Fellowship during Ph.D. Authors are also very grateful to Head, Department of Veterinary Surgery and Radiology and Department of Veterinary Pathology for providing the necessary facilities.

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