RESEARCH ARTICLE

Pathology and Diagnosis of Ocular Squamous Cell Carcinoma in Bovines

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Abstract

An investigation was carried out on 6 clinical cases of neoplasm of eye in bovines suspected of ocular squamous cell carcinoma (OSCC) which revealed the pathomorphological features and immunohistochemical expression of Pan-cytokeratin (Pan-CK), p53, epidermal growth factor receptor (EGFR) and p16, and confirmed OSCC in 3 out of 6 (50%) cases. Two cases were confirmatory in HF cattle and one in Murrah buffalo. Grossly, unilateral large firm cauliflower like growth, congested and reddish protruded mass of tissue covering the whole cornea resulted in blindness. Large nodular, hemorrhagic growth on limbus and lower eyelid with verrucous surface was also observed. OSCCs were classified into well, moderately and poorly differentiated types on the basis of histopathology and immunohistochemistry (IHC). Histopathological findings were distinctive keratin pearls with concentric layers of keratinization observed in well differentiated OSCC. Absence of epithelial pearls, although with numerous mitotic figures with evidences of anaplasia and neovascularization were observed in poorly differentiated OSCC, and moderate degree of keratinization at the center of neoplastic islets in moderately differentiated OSCC. The highest IHC expression was observed in Pan-CK, which confirmed the tumours of epithelial origin and EGFR was confirmatory for malignancy and degree of metastasis. Expression of Pan-CK in well differentiated OSCC revealed strong immunoreactivity towards Pan-CK. High immunoreactivity against EGFR with strong expression of EGFR was seen in moderately differentiated OSCC and mild immunoreaction in poorly differentiated OSCC. Mild immunoreaction for p16 was observed in one case. Thus, the neoplasms of eye can be confirmed as OSCC by immunoexpression of Pan-CK, EGFR, p16 and p53 in malignant tumours.

Key words: Anaplasia, EGFR, Epithelial pearls, Immunohistochemistry, Keratin, Pan-CK.

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Introduction

One of the biggest concerns causing decreased growth and production in bovines in field conditions is tumours. Squamous-cell carcinomas - malignant tumours of the stratified squamous epithelium - occur mostly in older animals (Vegad, 2007). Squamous cell carcinoma (SCC) is one of the most common cancers capable of metastatic spread and is observed in various forms across many animals and humans (Thomson, 2007; Tsujita and Plummer 2010; Yan *et al*., 2011). Important form of SCC is ocular squamous cell carcinoma (OSCC), commonly referred as "cancer eye". Squamous cell carcinoma is by far the most common and most frequently diagnosed tumour afflicting the bovine eye. It is a primary neoplasm of epithelial origin occurring in different ocular and periocular tissues including the palpebral skin, epithelial surfaces of the cornea and conjunctiva, third eyelid and limbus (Fornazari *et al*., 2017). The correlations in the breed wise incidence of eye cancer in cattle revealed highest incidence in HF crossbred followed by Jersey crossbred and non-descript cows (Radhakrishnan *et al*., 1999; Carvalho *et al*., 2005; Gharagozlou *et al*., 2007; Fornazari *et al*., 2017). Ocular squamous cell carcinomas were reported in Indian buffaloes also (Patel *et al*., 2009, Islam *et al*., 2017). This communication reports the pathomorphological and immunohistochemical study of OSCCs in 6 bovines.

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Materials and Methods

The present study was conducted from January to June 2022 in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Durg (Chhattisgarh), India to explicate the gross pathology,

histopathology and immunohistochemical alterations of ocular squamous cell carcinoma in bovines (n=6). Following gross examination, the tissue samples were collected in 10% neutral buffered formalin from clinical cases of ocular neoplasms presented and operated at Veterinary Clinical Complex of the College and Government Veterinary Hospitals of Durg and Rajnandgaon districts of Chhattisgarh for histopathological and immunohistochemical studies. Tumour biomarkers such as Tumour suppressor gene p53 & p16, Pancytokeratin (Pan-CK) and Epidermal Growth Factor Receptor (EGFR) in tissues were investigated by immunohistochemical technique.

Gross and Histopathological Examination

Gross morphological features of tumours like location, shape, colour and consistency of the tumour were recorded with gross lesions (Table 1). Tumours observed at eye were suspected to have been OSCC on the basis of gross pathology. Unique case identity was given to each sample for further detailed study. The formalin fixed tissues were cut into small pieces of 2-3 mm thickness, washed thoroughly in tap water overnight, dehydrated in graded acetone series, cleared with graded xylene and were embedded in paraffin wax. The paraffin embedded tissues were cut in 3-5 µ thickness. The sections were deparaffinised in xylene and stained with Haematoxylin and Eosin (H&E) stain, mounted with DPX (Bancroft and Stevens, 1990) and examined under light microscope for histological changes.

Histopathological scoring/grading was done according to Broder's system on the basis of histopathological findings such as degree of keratinization with evidence of formation of keratin pearls or cell nests, degree of anaplasia, invasion to the surrounding tissue and mitotic index (mitotic figures per high power field),), nuclear pleomorphism and inflammatory cell according to the method prescribed by Pugliese *et al*. (2014) and Fornazari *et al*. (2017). Histological malignancy grades of various tumours were also determined by sum of all histological findings.

Immunohistochemical Examination

Following preliminary diagnosis and histopathological examination of 6 OSCC cases, 4 selected tissue samples were processed for immunohistochemistry (IHC) to detect biomarkers such as Pan-CK, p53, EGFR, p16 and malignancy of tumours at Dr. Lal Path Labs, Path Vets Veterinary Diagnosis, Chittranjan Park, New Delhi. Commercial antibodies such as Anti-p53, Anti-p16 and Anti-EGFR procured from BioGenex Laboratories (Table 2), Fremont, California, USA were used in tumour masses. Pan-CK was detected by the use of CK-PAN procured from Zytomed System GmbH, Berlin, Germany.

Tissue Processing for Immunohistochemical Staining

Immunohistochemical labelling for all markers (p53, p16, Pan-CK and EGFR) was carried out on the Bench Mark Automated Staining System (Ventana Medical systems, Inc.). Antigen retrieval was performed for 60 min using Ventana Medical Systems Retrieval Solution CC1 according to the method prescribed by Fornazari *et al*. (2017). Slides were coated with Poly L-Lysine for staining and were examined under light microscope.

Table 1: Details of collected samples suspected of OSCC from different districts of Chhattisgarh and their histopathological features

Case ID	Age	Breed	Location of neoplasm	Surface appearance of neoplasm (Shape, color & consistency)	Histopathological findings
BovEC1	3 years	Non-descript	Nictitating membrane and lower eyelid of right eye	Soft, hyperemic pink colored mass	Not diagnosed as OSCC; absence of keratinization, less mitotic figures with no clear evidence of invasion.
BovEC2	5 years	Holstein Friesian	Lower eyelid, limbus and nictitating membrane of right eye	Soft, hyperemic pink colored neoplastic growth	Poorly differentiated OSCC; presence of individual keratinized cells, No keratin pearls; less invasion.
BovEC3	7 years	Holstein Friesian	Bulbar conjunctiva, cornea and eyelid of right eye	Large turgid, round, red irregular firm nodular mass, hemorrhagic and protruded out mass at bulbar conjunctiva	Moderately differentiated OSCC; presence of tumour island, moderate keratinization; with evidence of invasion
BovEC4	2 years	Kosli	Whole eyeball of left eye, extending deep into frontal and nasal sinus	Large oedematous and turgid, hard cauliflower like mass, poorly demarcated with verrucous surface	Well differentiated OSCC; presence of large cell nests, clearly differentiated; excessive keratinization.
BovEC5	5 years	Kosli	Whole eyeball of left eye, extending deep into frontal sinus	Large firm cauliflower like mass, congested and ulcerated growth	No clear evidence of OSCC; absence of keratinization, extensive hemorrhages.
BovEC6	4 years	Murrah buffalo	Bulbar conjunctiva, limbus and lower eyelid of left eye	Large nodular, hemorrhagic growth with verrucous surface.	Extensive fibrosis with large amount of fibroblasts; absence of keratin pearls; ballooning degeneration of cells; narrowing of keratinized epithelial layer with invasion of squamous cells to the surrounding fibrous connective tissue stroma

Immunohistochemical scoring was performed by estimating the percentage of positive cells and labeling intensity. The percentages of positive cells were scored as 0 (0% positive cells; non-immunoreactive), 1+ (Immunoreactivity in 1-25% cells), 2+ (Immunoreactivity in 26-50% cells), 3+ (Immunoreactivity in 51-75% cells), and 4+ (Immunoreactivity in 76-100% cells) as per the method described by Baghla *et al*. (2012). Staining intensity was scored as 0 (negative), 1+ (weak), 2+ (moderate), 3+ (strong), 4+ (extremely intense).

Results and Discussion

Gross Pathology

Gross examination of 6 unilateral neoplastic growths on bovine eyes suspected to have OSCC, revealed small, pink colored neoplastic growths, varied from soft to hard in consistency (Fig.1). Haemorrhagic, small, friable mass observed at lower eyelid in two cows, while in another case a large red colored hard protruded mass covering the whole cornea with severe hemorrhage resulting in blindness of cattle was observed in the right eye (Fig. 2). A large firm cauliflower like mass about 12 cm in size, extending deep into the frontal and nasal sinuses with verrucous surface was seen in left eye resulting in blindness in one more case (Fig. 3), while in another case of a cow tumourous mass at medial canthus of right eye extending towards limbus region was noted (Fig. 4). One case of ocular neoplasm (BovEC6) was recorded in female Murrah buffalo also with large nodular, hemorrhagic growth on limbus and lower eyelid of left eye with verrucous surface (Fig. 5a, 5b).

Gross pathological findings of neoplasms of eyes in most cases under study were in accordance with Pugliese *et al*. (2014). Verrucous surface of tumourous growth in eye was reported by Fornazari *et al*. (2017). Pinkish red coloured

Table 2: Tumour markers and primary antibodies used in immunohistochemical study

Tumour markers	Antibody	Clone	Catalogue no.	Make	Lab animal in which Ab raised with Ig class	Volume
Pan-cytokeratin (Pan-CK)	Cytokeratin Pan Plus	AE1 and AE3	MSG098	Zytomed system GmBH, Anhaltiner-stranbe 16, 14163 Berlin, Germany.	N/A	6 mL Conc., RTU
p53	Anti- p53	D07	AM239-10M	BioGenex Lab., Fremont, California (CA 94538) USA.	Mouse, Ig class: IgG1	10 mL, RTU
Epidermal Growth Factor Receptor (EGFR)	Anti-EGFR	Polycl-onal	AM335-10RE	BioGenex Lab., Fremont, California (CA 94538) USA	Rabbit, N/A	10 mL, RTU
p16	Anti- p16	G175-405	AM540-10M	BioGenex Lab., Fremont, California (CA 94538) USA	Mouse, Ig class: IgG	10 mL, RTU

RTU: Ready to Use, N/A: Not Available

Gross Pathology: Fig. 1: Hemorrhagic, small, friable mass observed at lower eyelid (Case: BovEC2); **Fig. 2:** Large red colored hard protruded mass covering the whole cornea with severe hemorrhage resulting in blindness in the right eye (Case: BovEC3); **Fig. 3:** Large firm cauliflower like mass, extending deep into the frontal and nasal sinuses, congested with verrucous surface was seen in left eye (Case: BovEC4); **Fig. 4:** Tumourous mass at medial canthus of right eye extending towards limbus region; **Fig. 5a:** Ocular neoplasm (BovEC6) in female murrah buffalo with large nodular, hemorrhagic growth on limbus and lower eyelid of left eye with verrucous surface; and **Fig. 5b:** Wart like tumourous growth spreading from lateral canthus to the whole cornea (Case ID: BovEC6).

turgid mass observed in the present study was also recorded by Lakshmi *et al*. (2020). Findings such as turgid masses scattered and poorly demarcated (BovEC4 and BovEC3) were also reported by Baniadam *et al*. (2010).

Histopathological Findings

Histopathological features of neoplasms of eyes suspected of OSCC are depicted in Table 1. Histopathological findings in OSCC were distinctive keratin pearls with more keratin deposition towards the center in one case (BovEC4, Fig. 6) and characteristic epithelial pearls with concentric layers of keratinization in another well differentiated OSCC (Fig. 7). Mineralization was also observed around the periphery of keratin pearls (Fig. 8). Numerous mitotic figures were also noticed with evidences of severe anaplasia (Fig. 9) and neovascularization mainly in poorly differentiated OSCC (Fig. 10). Typical neoplastic cells revealing anaplasia and numerous mitotic figures were observed (Fig. 11). One case (BovEC3) had a moderate degree of keratinization with keratin deposition at the center of neoplastic islets containing large anaplastic epithelial cells, multiple islands of tumourous squamous cells below the epidermal layer (Fig. 12). Cords of invasive tumour cells were clearly evident with mononuclear cell infiltration and keratinization at the center of neoplastic islets in OSCC. Large tumour islands with mononuclear cell infiltration were also observed in moderately differentiated OSCC (Fig. 12). Poorly differentiated OSCC were characterized with absence of epithelial pearls. Moderately differentiated OSCC were characterized with few small keratin pearls and multiple patches of keratin deposition in the form of cell nests (Fig. 13 a, b). Histological malignancy grade/score determined by sum of all histological findings (degree of keratinization + degree of anaplasia and invasiveness + mitotic figures) ranged from 2 to 8 in different cases of tumours diagnosed as OSCC.

Histopathological findings such as islands of tumourous epithelial cells, proliferation of fibroblasts, connective tissue and infiltration of lymphocytes and plasma cells were also reported earlier in OSCCs (Islam *et al*., 2017; Lakshmi *et al*., 2020; Sharma *et al*., 2020). Invasion of dermal stroma and highlighted keratinization with infiltration of neoplastic squamous cells observed in cases of OSCC in the present study were also reported by Fornazari *et al*. (2017). Histopathological findings of the present study such as anaplasia and neovascularization in squamous cell carcinomas were in accordance with Giri *et al*. (2011), Fornazari *et al*. (2017) and Sharma *et al*. (2020).

Immunohistochemical Findings

A total of 4 samples confirmed as OSCC on the basis of histopathology were further confirmed by immunoexpression and immunohistochemical scoring (IHS) (Table 3). OSCC were processed with all 4 tumour markers (Pancytokeratin, p53, EGFR and p16) for the detection of degree of epithelial malignancy and through immunoreaction of tumours to these markers.

OSCC revealed strong immunoexpression of Pan-CK, moderate immunoexpression for p53, EGFR, and negative to p16. Although in case of buffalo (BovEC6) mild immunohistochemical expression of p16 gene was observed, rest all cattle cases were non-immunoreactive to p16 marker. Immunohistochemical score (IHS) observed in Pan-CK, EGFR and p53 markers ranged from 1-3 (Table 3).

Immunohistochemical Expression of Pancytokeratin

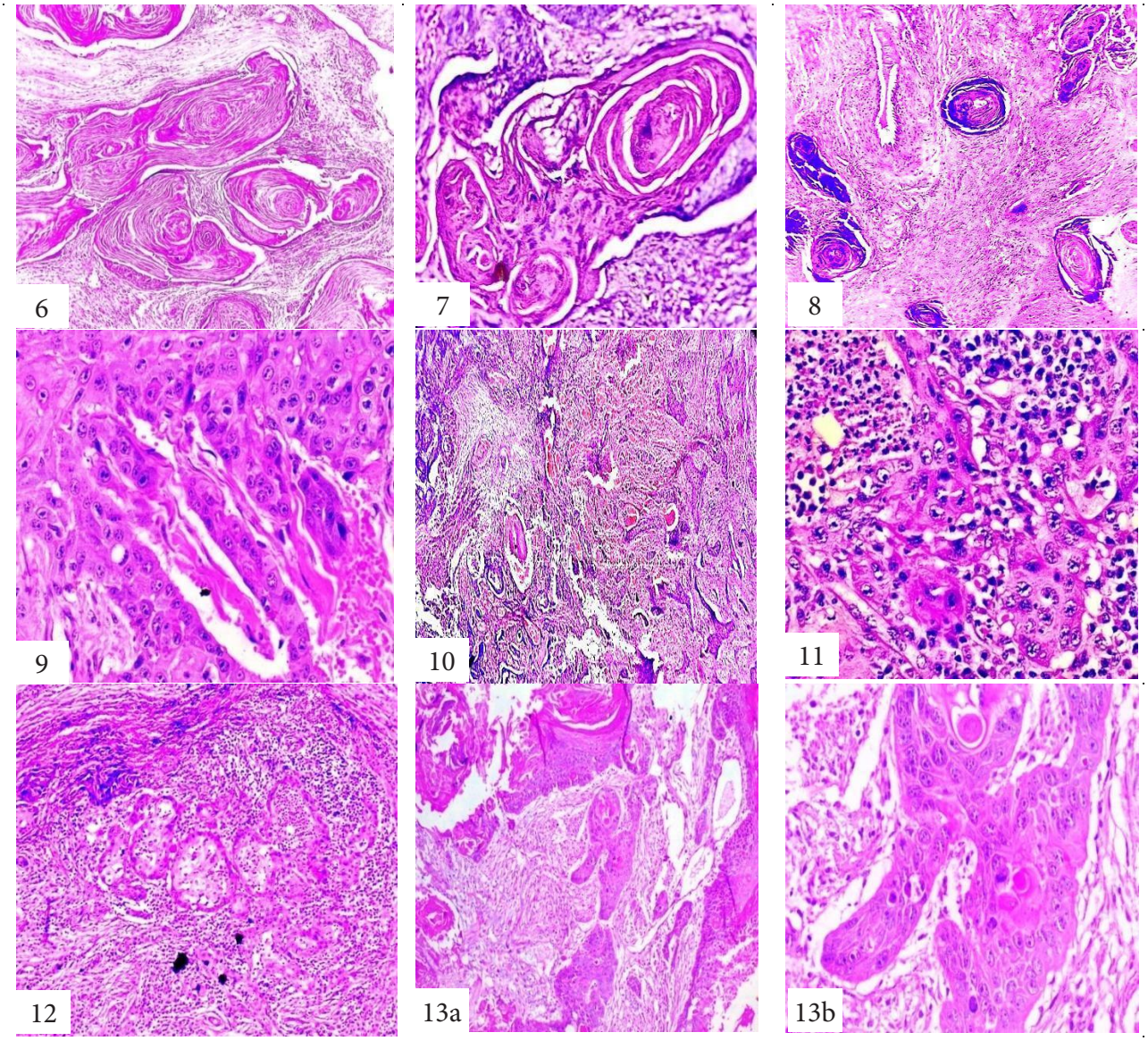
One of 6 cases (buffalo, BovEC6) revealed strong immunoreactivity of Pan-CK in the cell nests (keratin pearls) as well as in tumour islands (Fig. 14a,b). This indicated high Pan-CK immunoexpression in well differentiated OSCC. While another case (BovEC3) revealed strong Pan-CK immunoexpression in areas of keratinization in OSCC with 3+ score (Fig.15). Pan-CK immunoexpression in the epidermis with not much invasion to the surrounding tissue was observed in OSCC of Murrah buffalo (BovEC6; Fig. 16). Immunohistochemical findings of the present study were partially in accordance with the observations of Sharma *et al*. (2020). They depicted moderate cytoplasmic staining of Pan-CK in almost all neoplastic cells in 3 cases of undifferentiated OSCC, as were observed in the present study, with moderate immunoexpression of Pan-CK in OSCC (both poorly and moderately differentiated).

Immunohistochemical Expression of p53 Gene

One case (BovEC4) which was diagnosed as well differentiated OSCC by histopathology revealed mild to moderate immunopositive reaction towards p53 (Fig. 17) with 1+ score in IHS (Table 4). However, another ocular SCC (BovEC2) did not exhibit nuclear reactivity of p53 in the neoplastic cells of these tumours in spite of being histologically poorly differentiated OSCC. The concentration of p53 gene increases in response to DNA damage inside the nucleus of the cells thus responsible for immunoexpression in nucleus of tumourous cells. Nuclear staining of tumour cells around periphery of keratin pearls, sparing the region of keratinization with p53 tumour marker was also reported by Carvalho *et al*. (2005), Fornazari *et al*. (2017) and Sharma *et al*. (2020). High immunoreactivity observed in well differentiated OSCC in the present study differed from the findings of Sharma *et al*. (2020), who stated strong immunopositive reaction of p53 in poorly differentiated OSCC. Fornazari *et al*. (2017) observed intense positive immunostaining of p53 and expression mostly within outer epithelial layer of the cell nests. Inactivation of p53 is the possible mechanism for oncogenesis.

Immunohistochemical Expression of EGFR

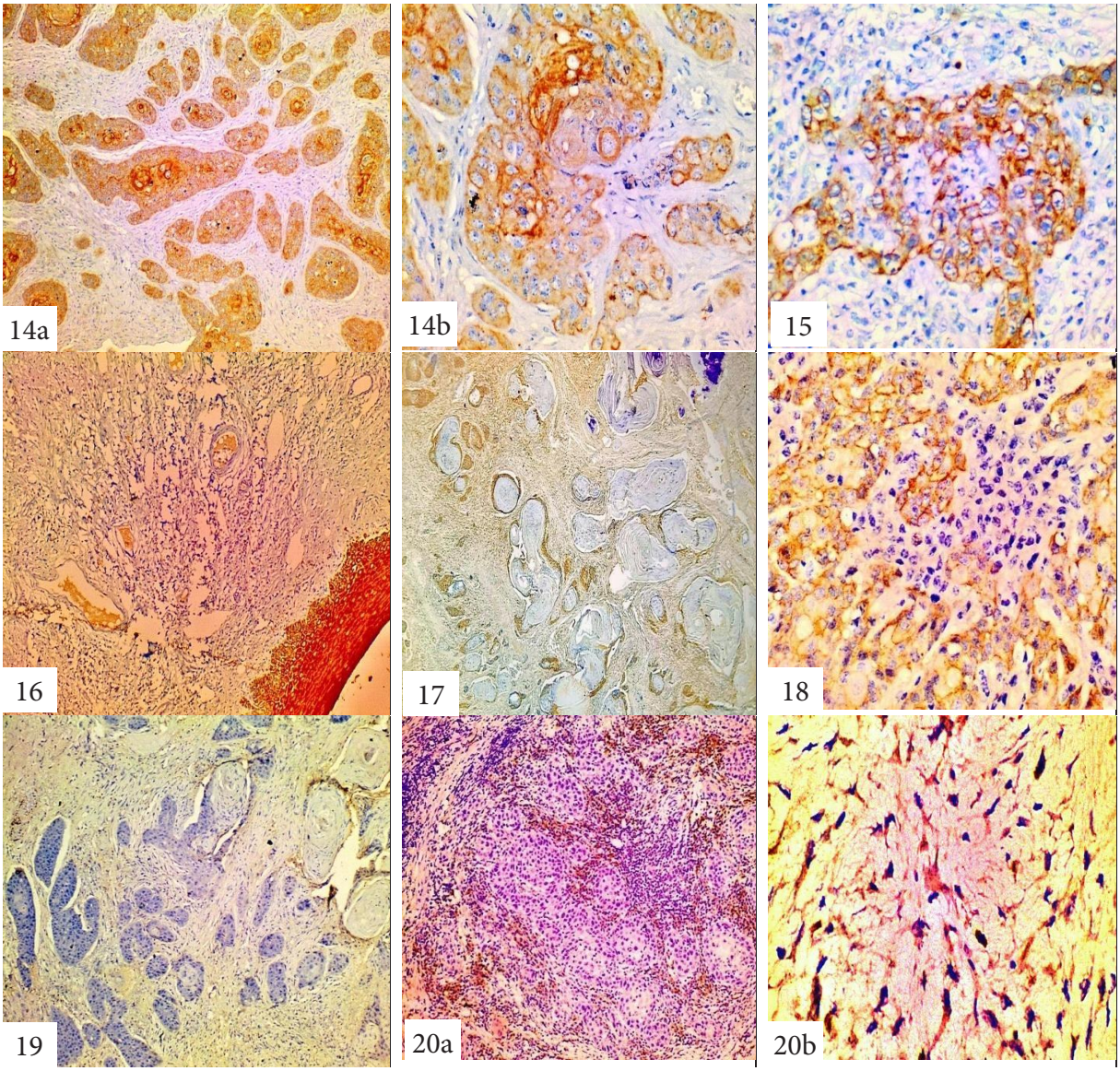
One case (BovEC3) exhibited high immunoreactivity against EGFR with the score of 3+ (Fig. 18). Strong immunohistochemical expression of EGFR in tumour islands in moderately differentiated OSCC and mild immunoreaction



Histopathology: Fig. 6: Well differentiated OSCC with multiple epithelial pearls (BovEC3; H& E X 400); **Fig. 7:** Well differentiated OSCC Showing characteristic epithelial pearls with concentric layers of keratinization (BovEC4; H&E X 400); **Fig. 8:** Well differentiated OSCC showing mineralization around the periphery of keratin pearls (BovEC4; H&E X 100); **Fig. 9:** Invasion of tumourous cells with mitotic figures in OSCC (BovEC3; H&E X 400); **Fig. 10:** Poorly differentiated OSCC; Illustrating neovascularization (BovEC2; H&E X 40); **Fig. 11:** Typical neoplastic cells revealing anaplasia and numerous mitotic figures in OSCC (BovEC3; H&E X 400); **Fig. 12:** Islands of tumourous squamous cells below the epidermal layer (BovEC3; H & E X 100); **Fig. 13a:** Multiple patches of keratin deposition in the form of cell nests (H&E X100); **Fig. 13b:** Higher magnification of Fig. 13 a) (H&E X 400).

Table 3: Immunohistochemical scoring of different tumour markers for bovine OSCC

Case ID	Histopathological diagnosis	p53 scoring	Pan-CK scoring	EGFR scoring	p16 scoring
BovEC2	Poorly differentiated SCC	0	0	0	0
BovEC3	Moderately differentiated SCC	2+	3+	3+	0
BovEC4	Well differentiated OSCC	1+	3+	1+	0
BovEC6	Fibrosis with evidence of poorly differentiated OSCC	1+	2+	1+	1+ (mild immuno-reactivity in <25% tumour cells)



Immunohistochemistry: Fig. 14a: Well differentiated OSCC; Strong immunoexpression of Pan-CK (BovEC4; IHC X 100); **Fig.14b:** Strong immunoexpression of Pan-CK in the cell nests (BovEC4, IHC X 400); **Fig. 15:** Pan-CK immunoexpression in areas of keratinization in OSCC (BovEC3, IHC X 400); **Fig. 16:** Poorly differentiated OSCC; Pan-CK immunoexpression in the epidermis in OSCC of Murrah buffalo (BovEC6, IHC X 100); **Fig. 17:** Well differentiated OSCC, mild to moderate immunoexpression of p53 with 1+ score (BovEC4, IHC X 100); **Fig. 18:** Moderate to strong immunoexpression of EGFR in OSCC with 3+ score (BovEC3); **Fig. 19:** Well differentiated OSCC, mild immunoreaction to EGFR at the periphery of epithelial pearls (arrow) (BovEC4, IHC X 100); **Fig. 20a:** Poorly differentiated OSCC, mild immunopositive reaction for p16 with 1+ score (BovEC6, IHC X 40); **Fig. 20b:**) Mild cytoplasmic p16 immunoreactivity in OSCC of Murrah buffalo with 1+ score (BovEC6, IHC X400).

of EGFR was observed mainly near to the epidermal layer in poorly differentiated OSCC in buffalo. OSCC showed positive immunoreaction towards EGFR although few samples were detected non-immunoreactive towards EGFR (BovEC4; Fig. 19). The present findings were in accordance with Lakshmi *et al*. (2020). They observed strong immunopositive reaction against EGFR in OSCC. High immunopositive reaction of EGFR confirmed the malignant tendencies of tumours of epithelial origin. Higher activity of EGFR signified the increased growth, invasiveness and metastasis of squamous cell carcinoma (Lakshmi *et al*., 2020).

Immunohistochemical Expression of p16

No immunoexpression for p16 marker was observed in 3 out of 4 cases (BovEC2, BovEC3, BovEC4) although 4th case of buffalo (BovEC6) exhibited mild immunopositive reaction for p16 with dark brown staining of nuclei and cytoplasm of tumour cells (Fig. 20 a, b) giving the IHS of 1+ (Table 4). The present findings were consistent with Fornazari *et al*. (2017), who reported negative immunoexpression of p16 in majority of cases of OSCC, however regionally intense positive immunostaining was observed in few cases of OSCC. Marinescu *et al*. (2016) reported positive

immunoexpression for p16 in poorly differentiated squamous cell carcinomas in the periphery as well as random in the islands of neoplastic cells. Further investigations are required to investigate p16 immunoexpression as a prognostic factor for bovines with cutaneous SCC as has been previously established for humans with the same tumour in the skin (Marinescu *et al*., 2016).

Conclusions

Grossly, ocular neoplastic growths among six cases studied were mostly pink colored varied from soft to hard in consistency although in one case large firm cauliflower like mass, extending deep into the frontal and nasal sinus, congested with verrucous surface was seen. Histopathologically, cell nests or keratin pearls with high degree of keratinization were observed in well differentiated ocular squamous cell carcinoma (OSCC) with anaplasia, numerous mitotic figures, tumour islands with severe inflammation, neovascularization, hemorrhages etc. Poorly differentiated OSCC was characterized with absence of keratin pearls although abundance of fibrous tissue was observed. Mild immunoexpression of p16 was observed in 1 case of poorly differentiated OSCC in a buffalo. Highest average immunohistochemical score was observed for Pan-Cytokeratin, which confirmed the tumours of epithelial origin and immunoexpression of EGFR was confirmatory for malignancy and degree of metastasis.

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References

Baghla, A., Choudhry, S., & Kataria, A. (2012). Immunohistochemical expression of cytokeratin 5/6 in gynaecological tumours. *International Journal of Pathology, 13*, 1-7.

Bancroft, H.D., & Stevens, A. (1990). *Theory and Practice of Histological Techniques*. Churchill Livingstone, Edinburgh.

Baniadam, A., Moezzi, N., & Mohammadian, B. (2010). Nasal squamous cell carcinoma in a cow. *Turkish Journal of Veterinary & Animal Sciences*, *34*(3), 303-305.

Carvalho, T., Vala, H., Pinto, C., Pinho, M., & Peleteiro, M. C. (2005). Immunohistochemical studies of epithelial cell proliferation and p53 mutation in bovine ocular squamous cell carcinoma. *Veterinary Pathology*, *42*(1), 66-73.

Fornazari, G.A., Kravetz, J., Kiupel, M., Sledge, D., De Barros Filho, I.R., & Montiani-Ferreira, F. (2017). Ocular squamous cell carcinoma in Holstein cows from the South of Brazil. *Veterinary World*, *10*(12), 1413.

Gharagozlou, M.J., Hekmati, P., & Ashrafihelan, J. (2007). A clinical and histopathological study of ocular neoplasms in dairy cattle. *Veterinarski Arhiv*, *77*(5), 409.

Giri, D.K., Kashyap, D.K., Dewangan, G., Tiwari, S.K., Ghosh, R.C., & Sinha, B. (2011). Squamous cell carcinoma of horn and its surgical management-a report of three cases. *International Journal of Livestock Sciences, 1*, 55-58.

Islam, S.T., Khurma, J., Wani, J.M., Ganaie, M.Y., Singh, A.K., Rashid, H., & Fayaz, I.B. (2017). Ocular squamous cell carcinoma in a female buffalo: A case report. *Journal of Entomology and Zoology Studies*, *5*(6), 795-796.

Lakshmi, M.P., Veena, P., Kumar, R.S., & RaniPrameela, D. (2020). Clinical, pathological and immunohistochemical studies on bovine eye cancer. *The Pharma Innovation Journal*, *9*, 353-355.

Marinescu, A., Stepan, A.E., Mărgăritescu, ClaudiU., Marinescu, A.M., Zăvoi, R.E., Simionescu, C.E., & Niculescu, M. (2016). P53, p16 and Ki67 immunoexpression in cutaneous squamous cell carcinoma and its precursor lesions. *Rome Journal of Morphology and Embryology*, *57*(2 Suppl), 691-696.

Patel, P.B., Mistry, J.N., Suthar, D.N., & Patel, J.B. (2009). Surgical management of ocular squamous cell carcinoma in buffalo calf. *IntasPolivet*, *10*(2), 293-294.

Pugliese, M., Mazzullo, G., Niutta, P.P., & Passantino, A. (2014). Bovine ocular squamous cellular carcinoma: a report of cases from the Caltagirone area, Italy. *Veterinarski Arhiv*, *84*(5), 449-457.

Radhakrishnan, C., William, B.J., Dharmaceelan, S., & Nagarajan, L. (1999). A successful treatment of bovine ocular squamous cell carcinoma by surgery and immunotherapy: A report of two cases. *Indian Veterinary Journal*, *76*(3), 245-246.

Sharma, S., Gupta, R.P., Jangir, B.L., Lather, D., & Hazari, R. (2020). Pathomorphological studies and immunohistochemical expression of p53 and pan-cytokeratin in bovine epithelial tumors. *Indian Journal of Veterinary Pathology, 44(1)*, 1-6.

Thomson, M. (2007). Squamous cell carcinoma of the nasal planum in cats and dogs. *Clinical Techniques in Small Animal Practice*, *22*(2), 42-45.

Tsujita, H., & Plummer, C.E. (2010). Bovine ocular squamous cell carcinoma. *Veterinary Clinics: Food Animal Practice*, *26*(3), 511-529.

Vegad, J.L. (2007). Neoplasia. In: *A Textbook of Veterinary General Pathology*, 2nd revised and enlarged edn. International Book Distributing Co. Lucknow, India, pp: 281, 376-377.

Yan, W., Wistuba, I.I., Emmert-Buck, M.R., & Erickson, H.S. (2011). Squamous cell carcinoma–similarities and differences among anatomical sites. *American Journal of Cancer Research*, *1*(3), 275.