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Clinical, pathological and immunohistochemical studies on bovine eye cancer

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Abstract

The present clinical study was conducted to study incidence, clinical symptoms, histopathological and immunohistochemical findings of bovine eye cancer. A total of 36 cattle aged between 1 to 8 years were involved in this study. Incidence of eye cancer in bovines among a total number of bovine cases was recorded over a period of one year. The incidence of eye cancer in cattle was found to be high in age group of above 5 years followed by 2-5 years. The correlations in the breed wise incidence of eye cancer in cattle revealed highest incidence of holstein friesian crossbred cows followed by jersey crossbred cows and non-descript cows. Histopathologically, all cases were evident of squamous cell carcinoma. Immunohistochemical studies were conducted to know the rate of proliferation of bovine eye cancer. Immunopositive reaction was observed against VEGF and EGFR in most of the bovine eye cancer.

Keywords: Bovine eye cancer, histopathological, immunohistochemical studies

Introduction

Bovine ocular squamous cell carcinoma also called cancer eye, represents the most economically important neoplasm in large animals. Hereditary factors, environmental factors (eg: latitude, altitude, exposure to sunlight), lack of eyelid pigmentation, age and dietary habits have all been reported to play a role in the etiopathogenesis of bovine ocular squamous cell carcinoma. In addition, in cattle the etiology has been linked to a number of viral agents, especially bovine papilloma virus and bovine herpes virus type 1 and 5. Nevertheless, ultraviolet light, viruses and circumocular pigmentation are the major epidemiologic risk factors for the development of the tumor. The aim of the present study was to describe the clinical, macroscopic, histopathological and immunohistochemical findings of bovine eye cancer.

Materials and methods

A total of 36 cattle aged between 1 to 8 years were involved in this study. Incidence of eye cancer in bovines among a total number of bovine cases was recorded over a period of one year. The data regarding the incidence of bovine eye cancer were collected and variables like age, breed, sex, location of eye cancer and clinical symptoms were also recorded and analyzed. Diagnosis was done based on the clinical signs and histopathological findings.

Histopathological studies

Representative tissue samples were collected under local anaesthesia for histopathological studies. These samples were fixed in 10% buffered formalin and were processed routinely for histopathological examination. The sections of 5 micron thickness from embedded paraffin block were made and stained with Haematoxylin & Eosin (H&E). The sections were examined microscopically for histological changes.

Immunohistochemical studies

Paraffin sections of bovine ocular squamous cell carcinoma tissue samples were cut at 3 – 4 µm thickness mounted on APES (Amino Propyl Ethoxy Sialine) coated slides and incubated overnight at 37°C. These slides were subjected to immunohistochemistry protocol.

Procedure

- Sections were deparaffinized by passing through xylene 2 changes 15 minutes each and dipped in absolute alcohol 2 changes to remove xylene. Washed under running tap water

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for 10 min. Rinsed in distilled water for 5 min.

- Kept in Tris EDTA buffer (pH-9.0) and hot water bath treatment was given for 20 min at 100°C to retrieve the antigenic sites.
- Cooled to the room temperature, kept in the distilled water 5 min and in Tris buffer saline for 5 min.
- The slides were kept in the humid chamber and in the peroxidase block solution (3% hydrogen peroxides – freshly prepared) for 30 min to block the endogenous peroxidase.
- Washed in Tris buffer saline for 3 times 5 min each. The power block solution using 1.5% BSA was poured on tissue section and kept for 30 min. Primary antibodies (VEGF, EGF and Ki67) were added on the sections and slides were kept at room temperature for 2 hrs or at 4°C overnight.
- Washed in Tris buffer saline for 5 min each in 3 changes.
- Secondary antibody with HRP was added and kept for 1 hr. Washed in Tris buffer saline for 5 min each in 3 changes.
- From Diaminobenzidine (DAB), the working coloring reagent was prepared by adding one drop of DAB to 1 ml of substrate. The sections were kept in the coloring reagent for 30 min.
- Washed in Tris buffer saline for 2 min for 2 times and in tap water for 10 min. Stained with Harris haemotoxylin for 1 min. Washed in tap water for 5 min. Then air dried and mounted with DPX.
- Development of brown color in the slide indicates positive reaction whereas blue color development was due to staining with haemotoxylin.

Results

Data pertaining to age, breed, sex, location of papillomas and clinical symptoms were shown in Table 1 to 6.

Table 1: Incidence of eye cancer in different age groups of cattle (n=36)

S. No.	Age of animals	No. of animals	Percentage
1	2-5 years	12	33.33
2	Above 5 years	24	66.67
	Total	36	100

Table 2: Incidence of eye cancer in different breeds of cattle (n=36)

S. No.	Breed	No. of animals	Percentage
1	HF Cross bred	27	75.00
2	Jersey cross bred	6	16.66
3	ND cows	3	08.33
	Total	36	100

Table 3: Sex wise incidence of eye cancer in cattle (n=36)

S. No.	Sex of animals	No. of animals	Percentage
1	Males	2	5.55
2	Females	34	94.45
	Total	36	100

Table 4: Location of eye cancer in cattle (n=36)

S. No.	Location of warts	No. of animals	Percentage
1	Nictitating membrane	14	38.89
2	Lower eyelid	7	19.44
3	Lower eyelid and nictitating membrane	6	16.66
4	Eyeball	3	8.33

5	Upper eyelid	2	5.55
6	Upper and lower eyelid	2	5.55
7	Upper eyelid and medial canthus	2	5.55
	Total	36	100

Table 5: Showing involvement of eye in cattle (n=36)

S. No.	Eye affected	No. of animals	Percentage
1	Right eye	19	52.78
2	Left eye	17	47.22
	Total	36	100

Table 6: Types of eye cancer in cattle (n=36)

S. No.	Types of eye cancer	No. of animals	Percentage
1	Cauliflower like	24	66.66
2	Papilloma like	11	30.55
3	Papule like	1	2.78
	Total	36	100

The gross lesions appeared as raised, fleshy, pink to red in color and ulcerated irregular growths involving nictitating membrane. The average width of tumorous growths ranged from 0.5 cm to 10 cms. Some growths were extensive involving eye and adnexa due to which animal was unable to open the eyes. Most of the tumors were sessile or non-pedunculated, irregular or cauliflower like masses.

Histologically, all cases were evident of squamous cell carcinoma. The sections revealed arrangement of cells in broad sheets and gradually accumulated in concentric manner forming cell nests. Inter cellular bridges with typical of normal prickle cells is evident. Hyperchromatic, pleomorphic and pyknotic nuclei with mitotic figures of varying nature were present. At few places, elongated spindle shaped cells with fibroblasts proliferation, infiltration of lymphocytes and plasma cells are also noted. In few growths the lesions revealed pigmented cells in the epidermis with large cells, slightly fusiform in shape (Fig 1).

Immunohistochemical studies were carried out by using VEGF and EGFR stains. Vascular Endothelial Growth Factor (VEGF) showed mild immunopositive reaction (Fig 2) and Epidermal Growth Factor Receptor (EGFR) revealed strong immunopositive reaction against EGFR (Fig 3).

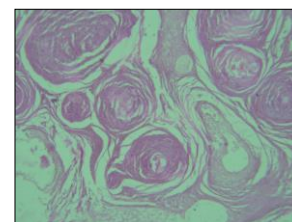


Fig 1: Growth collected from left eye for HF crossbred cow revealed large cell nests with few hyperchromatic nuclei with considerable mitotic figures.

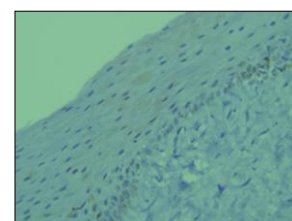


Fig 2: Showing - VEGF - Mild reaction is observed

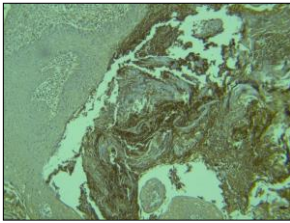


Fig 3: EGFR – strong positive reaction is observed

Discussion

The incidence of eye cancer in cattle was found to be high in age group of above 5 years followed by 2-5 years (Heeney and Valli 1985)^[7] who observed eye cancer in above 5 years aged cattle. This could be due to the allowing of adult animals to outside leading to exposure to radiation or tying the animals outside.

The correlations in the breed wise incidence of eye cancer in cattle revealed highest incidence of holstein friesian crossbred cows followed by jersey crossbred cows and non-descript cows (Carvalho *et al.* 2005, Gharagozlou *et al.* 2007, Daryoush *et al.* 2011, Fornazari *et al.* 2017 in holstein cattle, Radhakrishnan *et al.* (1999)^[2, 6, 3, 5, 11] and Fazili *et al.* (2001)^[4] in jersey cows. However, no incidence of eye cancer was recorded in buffaloes in this study. The availability of different breeds in a particular geographical location would predispose certain breeds to get affected. The incidence was higher in crossbred animals since crossbred population is high in this area. This shows higher susceptibility of crossbred cattle in comparison with indigenous cattle.

The incidence of bovine eye cancer was found to be high in females (Gharagozlou *et al.* 2007 and Schulz and Anderson 2010)^[6]. The reason might be the female cattle usually under stress factors such as gestation, lactation and progression in age. On the other hand, male cattle are usually directed to fattening and meat production and are mostly slaughtered at the age of 2 years or less.

In the present study, maximum number of animals was having eye cancer on nictitating membrane, followed by lower eyelid, concurring with the findings of Carvalho *et al.* (2005)^[2] and Kalirajan and Senthilkumar (2016)^[8]. However, eye cancer involving lower eyelid and nictitating membrane, eyeball, upper and lower eyelids and medial canthus of the eye were also recorded in the present study (Panchbhai *et al.* 1989)^[9]. In our study, most of the eye cancer in cattle was observed on right eye followed by left eye (Fornazari *et al.* 2017)^[5].

Diagnosis of bovine eye cancer is not always a hard task as the lesions can always be seen as red, ulcerated, irregular masses arising from the various parts of eye. Confirmatory diagnosis of eye cancer was achieved by histopathological examination of the tumor mass. Histologically, all cases were diagnosed as squamous cell carcinoma in our study (Fazili *et al.* 2001, Carvalho *et al.* 2005, Azarabad *et al.* 2011)^[4, 2, 1].

VEGF is a potent angiogenic factor, produced by a variety of cell types, including keratinocytes, endothelial cells, macrophages, mast cells and fibroblasts and is involved in several types of tumors, where it has been shown to influence both tumor neovascularization and dissemination. In the present study, mild expression of VEGF was observed suggesting a possible role in development of eye cancer through angiogenesis.

Epidermal Growth factor receptor (EGFR) is a key factor in epithelial malignancies, and its activity enhances tumor growth, invasion and metastasis. EGFR plays an important

role in maintaining normal cell function, dysregulation of EGFR signaling pathways contributes to the development of malignancy via effects on cell cycle progression, inhibition of apoptosis, induction of angiogenesis and promotion of tumor cell motility and metastasis. In our study, EGFR immunopositive reaction was noticed in different layers of epidermis in the cytoplasm of fibroblasts present in dermis which established that dysregulation of EGFR receptor signaling pathway is associated with the development and progression of malignancy.

In this study, the authors described clinical and pathological findings of bovine eye cancer. Immunohistochemical studies like VEGF and EGFR were very useful to detect cellular proliferation in the bovine eye cancer.

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