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Expression of PCNA, MMP-9 and P53 in Bovine Ocular Squamous Cell Carcinomas: an Immunohistochemical Study

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Abstract

In this study, we aimed to compare and correlate the PCNA, MMP-9 and p53 expressions and differentiation degree of bovine ocular squamous cell carcinomas (BOSCCs) by immunohistochemical methods. The material of this study was composed of BOSCC biopsy samples taken from 30 cattle brought to our department. Tissue samples were fixed in 10% buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin & eosin in order to detect histopathological changes. Sections were examined and photographed under a light microscope. Avidin-biotin-peroxidase method was used for immunohistochemistry. Macroscopically the masses were nodular to cauliflower-like shaped. The surfaces of the masses were highly hemorrhagic and ulcerative, and sometimes covered with a purulent discharge. Histopathologically, we defined cases with excessive and large numbers of keratin pearls, large tumoral islands, and evident squamous differentiation as well-differentiated. Cases with smaller tumoral islets, decreased number and size of keratin pearls, but higher number of poorly-differentiated cells compared to well-differentiated cases were defined as moderately-differentiated. Tumors in which keratinization was either absent or formed in individual cells were classified as poorly-differentiated. Statistical analysis revealed that there was no significant difference between well, moderately, and poorly differentiated tumors in terms of PCNA and MMP-9 expressions, but we found that the increase in p53 expression negatively correlated with the degree of differentiation of the tumor. In conclusion, we think that p53 can be a useful marker in determining the prognosis of BOSCCs.

Keywords: cattle, ocular squamous cell carcinoma, MMP-9, p53, PCNA.

Introduction

Bovine ocular squamous cell carcinoma (BOSCC), also known as 'cancer eye', is the most common neoplasm of the eye in cattle and causes serious economic problems.[1,2] Hereditary factors, environmental factors such as latitude, altitude, and exposure to sunlight (ultraviolet light), viruses (bovine papillomavirus and bovine herpesvirus types 1 and 5), lack of eyelid pigmentation, age and dietary habits play an important role in the etiopathogenesis of BOSCC.[3-5] The tumor is mostly detected in Hereford, Holstein, and Simmental cattle breeds; the incidence is more common in the age of 3 to 7 years; and the tumor has no sex predisposition.[6-8] BOSCC, which originates from the stratum spinosum cells of stratified squamous epithelium, can occur in different ocular and periocular tissues including the palpebral skin, epithelial surfaces of the cornea and conjunctiva, third eyelid, and limbus.[8,9] The tumor is typically ulcerative, firm, lobular, and has a cauliflower-like appearance, and when seen with purulent panophthalmitis, it can be covered with a mucopurulent exudate.[7,10] Histologically, BOSCC ranges from well-differentiated to undifferentiated and has a highly variable degree of invasiveness.[11,12] Well-differentiated tumors contain concentric lamellations of glob cornes, while poorly differentiated tumors are highly anaplastic and have small hyperchromatic cells without

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keratinization.[4] BOSCC primarily metastasizes to the parotid lymph node and secondarily to the salivary glands, organs such as the lungs, liver, kidneys, and rarely to the brain.[13]

Proliferating cell nuclear antigen (PCNA) is a 36 kDa non-histonic nuclear peptide essential for DNA replication and is considered as a reliable marker of cell proliferation in various tumors.[14-16] There is a correlation between PCNA expression and the degree of malignancy and the progression of cancer.[15] Although still controversial, PCNA overexpression has been associated with the recurrence rate and prognosis of oral SCC in dogs.[14] Matrix metalloproteinases (MMPs) are a multigene family of ubiquitous zinc endoproteinases involved in the degradation of the extracellular matrix (ECM) specific components and basement membrane, promoting invasive and metastatic actions of tumoral cells.[17-19] The expression and activity of MMPs have been shown to increase in SCCs of the head and neck of humans, and this increase was correlated with invasion, metastasis, and poor prognosis.[20,21] p53, also known as protein 53 or tumor protein 53, is a tumor suppressor protein encoded by the TP53 gene.[22] p53 has important functions in the regulation of cellular development and proliferation, besides, it monitors the integrity of DNA and, thus, is also called the guardian of the genome.[23] p53 protein levels increase temporarily as a response to DNA damage and this increase leads to cellular repair processes or triggering of apoptosis.[24] Mutations in p53 gene (by deletion, point mutation or rearrangement) lead to rapid selection of malignant clones, uncontrolled cell proliferation, and inhibition of apoptosis.[12,24,25] Squamous cell carcinomas commonly have the p53 mutation. p53 immunoreactivity has particulary been detected in non-pigmented skin SCCs of animals (cattle, horses, cats, and dogs) exposed to UV radiation.[9,24] In this study, we aimed to evaluate the PCNA, MMP-9, and p53 expressions in BOSCCs immunohistochemically and investigate any possible correlation between expression levels and the differentiation degree of these tumors. Whether these immunohistochemical markers are useful in determining the prognosis of the tumor is discussed in the light of the data obtained from our study.

Materials and Methods

Animals

In this study, 30 biopsy samples taken from BOSCCs and referred to our department for diagnosis between 2016 and 2021 were used. This study was designed as a retrospective study using archival materials. Information on the breed, age, sex, of the animals and localization, gross findings, and differentiation degree of the tumors are given in Table 1.

Table 1: Information on the breed, age, and sex of the animals and localization, gross findings, and differentiation degree of 30 bovine ocular squamous cell carcinomas (BOSCCs)

Breed	Age (years)	Female/Male	Right/left eye	Tumor location	Gross findings	Differentiation degree
Simmental (n=25)	4-9 (mean: 6.12)	24 F, 1 M	10 R, 15 L	Whole eye (n=12) Lateral canthus (n=4) Upper eyelid (n=4) Lower eyelid (n=4) Third eyelid (n=1)	Cauliflower-like appearance, hemorrhagic (n=9) Panophtalmitis (n=12) Nodular, hemorrhagic (n=4)	Well-differentiated (n=7) Moderately differentiated (n=8) Poorly differentiated (n=10)
Simmental cross (n=2)	5-6 (mean: 5.5)	2 F	1 R, 1 L	Whole eye (n=1) Upper eyelid (n=1)	Nodular, hemorrhagic (n=1) Panophtalmitis (n=1)	Well-differentiated (n=1) Moderately differentiated (n=1)
Zavot (n=2)	7 (mean: 7)	2 F	2 R	Whole eye (n=2)	Cauliflower-like appearance, hemorrhagic (n=1) Panophtalmitis (n=1)	Well-differentiated (n=2)
Hereford (n=1)	5 (mean: 5)	1 F	1 R	Lower eyelid (n=1)	Cauliflower-like appearance, hemorrhagic (n=1)	Moderately differentiated (n=1)

F: female, M: male, L: Left, R: Right

Ethical Approval

The ethics committee report of this study was obtained from Kafkas University Animal Experiments Local Ethics Committee (authorization number: KAU-HADYEK-2020/171).

Histopathological Investigations

Tissue samples were fixed in 10% buffered formalin, processed routinely, and sections were stained with hematoxylin&eosin (H&E). All slides were examined under a light microscope (Olympus Bx53) and photographed via the Cell^P program (Olympus Soft Imaging Solutions GmbH, [3,4]).

In determining the differentiation degrees of all BOSCC cases, the basic criteria suggested by Carvalho et al.[12], Gharagozlou et al.[26] and Sözmen et al.[2] were taken into consideration. Tumors with excessive and large numbers of keratin pearls, large tumoral islands, and evident squamous differentiation were defined as well-differentiated (WD), while tumors with small-to-medium sized keratin pearls, medium-sized tumoral islands, and an increase in poorly differentiated cells were defined as moderately differentiated (MD). Tumors with either no keratinization or keratinization in few individual cells, with very small tumor islands and highly pronounced pleomorphism were defined as poorly differentiated (PD).

Immunohistochemical Investigations

Avidin-biotin-peroxidase method was used for immunohistochemistry. For immunohistochemical staining, 4 µm sections were transferred to poly-L-lysine coated slides.

The slides were then deparaffinized and rehydrated in graded alcohols. In order to prevent endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide solution in phosphate buffered saline (PBS) for 15 min., and then were boiled in citrate buffer solution (pH 6) for 25 min. in microwave oven (at 800 watts) for antigen retrieval. Nonspecific staining was prevented by incubating the slides for 10 min. with non-immune serum (Thermo Scientific Histostain-Plus IHC Kit, HRP, broad spectrum, REF: TP-125-HL). Slides were later incubated with diluted antibodies (PCNA: Santa Cruz, sc-56, dilution ratio: 1:100, MMP-9: Santa Cruz, sc-393859, dilution ratio: 1:100, p53: Novus Bio, SPM590, dilution ratio: 1:100) overnight at +4 °C. The sections were washed 3 times in PBS solution for 5 min., and biotinylated secondary antibody (Thermo Scientific, Histostain-Plus IHC Kit) was applied for 10 min. After washing in PBS (3-5 min.), all sections were incubated with peroxidase-bound streptavidin (Thermo Scientific, Histostain-Plus IHC Kit) for 10 min. A solution of 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Thermo Scientific, REF: TA-125-HD) was used for 15 min. as chromogen. The sections were treated with Mayer's hematoxylin for 30 seconds and washed in running water for 5 min. For negative control, primary antibodies were omitted and the slides were treated with diluted normal serum. All steps, except for incubation with primary antibody, were carried out at room temperature. The slides were finally examined under a light microscope and photographed as described above.

The PCNA, MMP, and p53 immunoreactivities were scored by counting the number of positively stained cells in areas with highest immunostaining intensity. For each sample, five different areas were examined under x200 magnification. The number of positively stained cells in each area was recorded and the average was used for statistical analysis.

Statistical analysis

The groups obtained according to the differentiation degrees showed normal distribution according to the Kolmogorov-Smirnov test. One-way ANOVA was used for group comparison. Since the homogeneity test of variances in the groups was statistically significant ($P < 0.05$), the degrees of differentiation were compared with the Post-Hoc Games-Howell test. Statistical analyzes were performed using the SPSS® (version 20.0, Chicago, IL, USA) program. Differences obtained among the groups after statistical analysis were considered significant at $P < 0.05$.

Results

Clinical findings

Information about the animals having BOSCC and the tumors are presented in Table 1. The most significant anamnesis obtained from the patient owners was that visual difficulties caused by tumoral masses resulted in loss of productivity. Some masses had quickly reached significant sizes in very short time frames, and this period was quite prolonged in some masses.

Gross findings

We determined that the size of the masses varied from chickpea size to tennis ball size. Some of the masses were nodular and had cauliflower-like appearance. The surfaces of the masses were highly hemorrhagic andulcerative, and sometimes covered with a purulent discharge. The masses frequently spread to the whole eye, causing complete deformation and atrophy of the eye as a result of the pressure they created (Figure 1). As a result of the inspection and palpation examination performed before the surgical operation, we did not find distant or adjacent tissue metastases.

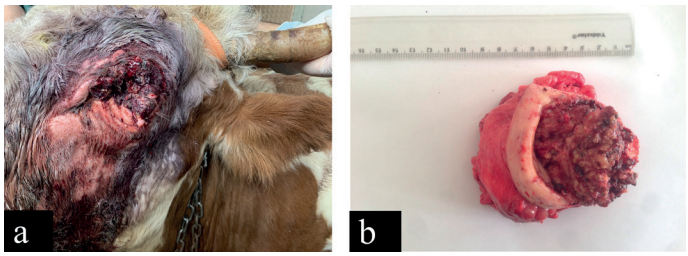

Figure 1: a) Macroscopic view of a cauliflower-like appearing mass with hemorrhage spreading over the whole eye. b) The appearance of the mass after surgery.

Histopathological findings

We defined cases with excessive and large numbers of ker-

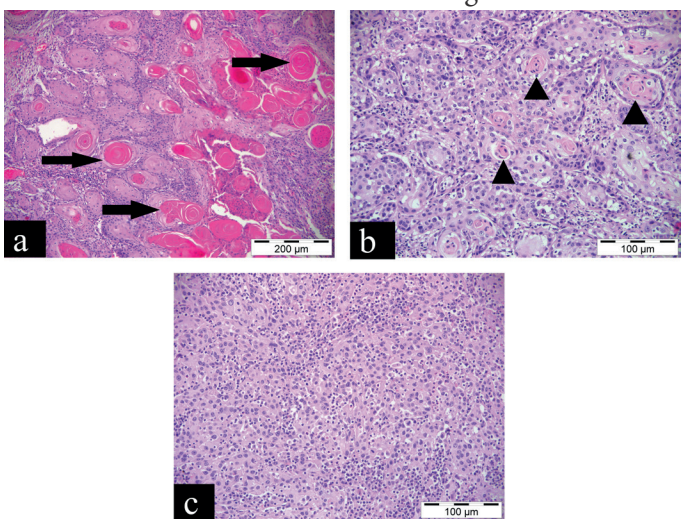

Figure 2: a: Well-differentiated bovine ocular squamous cell carcinoma (BOSCC). Numerous and large keratin pearls (arrows) H&E, Bar= 200 µm. b: Moderately differentiated BOSCC. A small number of medium or small sized keratin pearls (arrowheads), H&E, Bar= 100 µm. c: Poorly differentiated BOSCC. Tumor cells with pronounced pleomorphism and no keratinization, H&E, Bar= 100 µm.

atin pearls, large tumoral islands, and evident squamous differentiation were defined as WD. In MD cases, we found that the number and size of keratin pearls decreased compared to WD cases. In addition, we observed that tumoral islets were smaller in these cases, similar to keratin pearls, and the number of poorly differentiated tumor cells increased. In PD cases, we observed that keratinization was either absent or formed in individual cells. We found that tumor islets are quite small compared to MD and WD cases, and pleomorphism increased considerably (Figure 2).

Onion membrane-like keratin pearls were detected in the middle of tumoral islets or trabeculae formed by neoplastic cells with pale eosinophilic cytoplasm and vesicular nucleus in WD and MD groups. Tumoral cells had enlarged and prominent nucleolus in PD groups. In addition, an increase in the number of nucleoli (up to 5) was found in PD groups. Significant hyperchromasia of nuclei was detected in some tumoral areas in PD groups. An increase was determined in nucleus:cytoplasm ratio in PD groups. The presence of bizarre giant cells, abnormal mitotic figures, apoptotic cells, and megakaryocytes was remarkable in PD groups. Intercellular bridges were evident among the tumor cells in WD groups. Additionally, the presence of dyskeratotic cells were observed in WD, MD and PD groups. In all groups inflammatory cells, mostly composed of lymphocytes, plasma cells, and histiocytes, were detected among the tumor islands. In addition hemorrhage, necrosis, and neutrophil infiltration were also observed especially on the outer surface of the tumoral masses.

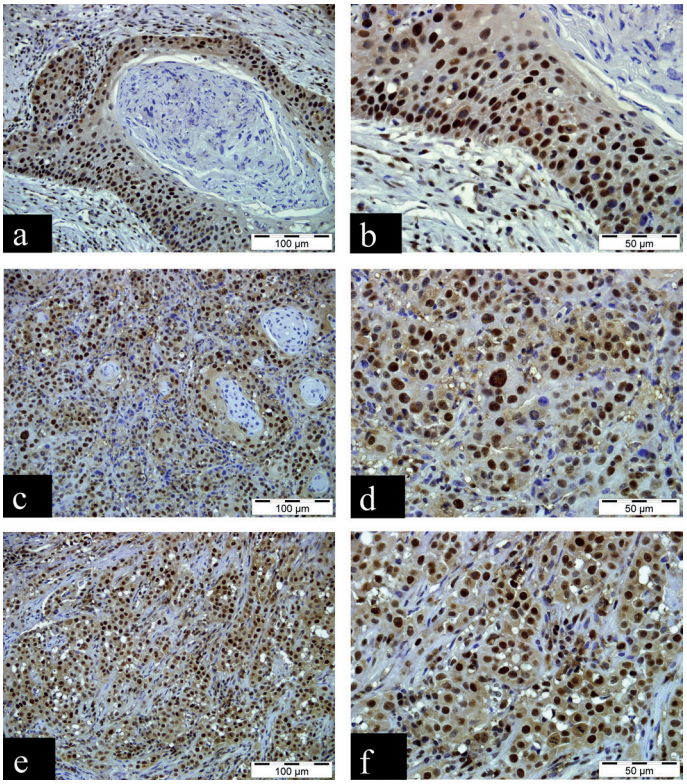

Figure 3: PCNA, IHC a) Well-differentiated bovine ocular squamous cell carcinoma (BOSCC). Immune-positive reactions in neoplastic cells around keratin pearls, Bar=100 µm. b) Higher magnification of Figure 3a. Dark brown positive expressions in the nucleus, Bar=50 µm. c) Moderately differentiated BOSCC. Immunoreactivity in tumoral cells around small to medium sized keratin pearls, Bar=100 µm. d) Higher magnification of Figure 3c. Strong dark brown positive expressions in the nucleus, Bar=50 µm. e) Poorly differentiated BOSCC. *Diffuse immune-positive expressions in the poorly differentiated tumoral areas, Bar=100 µm. f) Higher magnification of Figure 3e. Strong positive expressions in the nucleus, Bar=50 µm.*

Immunohistochemical findings

Mean ± SE values of all groups are given in Table 2. Tumors of all differentiation degrees were immunopositive for PCNA, MMP-9, and p53 expressions. We did not detect any significant difference in PCNA expression between WD, MD, and PD tumors. Tumors from all differentiation degrees, showed PCNA positivity particularly in the nuclei of tumor cells. In WD and MD tumors, the intensity of PCNA reaction was particularly strong in tumoral cells located at the periphery of the tumoral islands surrounding the keratin pearls. In PD tumors, PCNA positivity was particularly significant in tumoral cells with pronounced pleomorphism (Figure 3). Similar to the PCNA results, no statistically significant difference was observed among the WD, MD, and PD tumors in terms of MMP-9 expression.

Table 2: Differentiation degree of bovine ocular squamous cell carcinomas (BOSCCs) and the average number of positively stained tumor cells in five areas examined under x200 magnification with PCNA, MMP9 and p53 immunostainings. Values are presented as mean ± SE.

Groups	PCNA	MMP9	p53
Well-differentiated (n=10)	397.38 ± 25.25	304.56 ± 14.14	141.63 ± 19.32[a]
Moderately differentiated (n=10)	451.60 ± 35.96	365.44 ± 11.56	249.13 ± 18.74[b]
Poorly differentiated (n=10)	456.43 ± 6.12	368.60 ± 38.52	391.44 ± 36.15[c]
P value	NS	NS	<0.001

a,b,c: Different letters in each column indicate significant difference among the groups. a-b: P=0.004, a-c: P<0.001, b-c: P=0.012. NS: Non-significant

We detected intracytoplasmic MMP-9 positivity in neoplastic cells located in the outer layer of tumoral islands in WD and MD tumors. Granular intracytoplasmic MMP-9 expression was remarkable in pleomorphic cells in PD tumors (Figure 4). Unlike the PCNA and MMP-9 immunostaining results, the number of p53-positive cells increased significantly as the degree of differentiation increased. We observed that p53 expression in WD and MD tumors was particularly strong in the neoplastic cells located in the periphery of tumor islands/cords. No p53 expression was

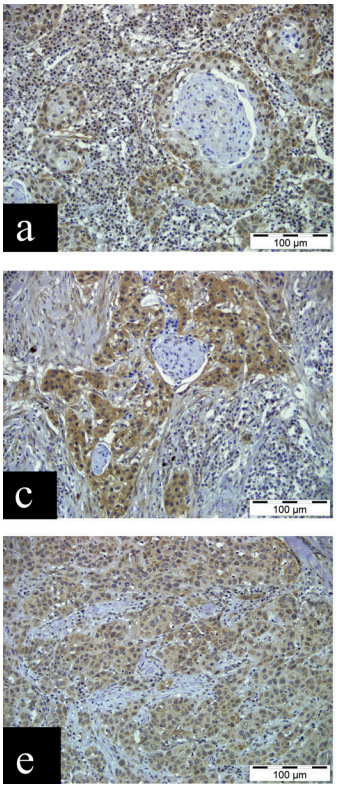

Figure 4: MMP-9, IHC a: Well-differentiated bovine ocular squamous cell carcinoma (BOSCC). Intracytoplasmic immune-positive reactions in neoplastic cells around keratin pearls, Bar=100 µm. b: Higher magnification of Figure 4a. Yellow-brown positive expressions in the cytoplasm, Bar=50 µm. c: Moderately differentiated BOSCC. Immune-positive reactions in tumoral cells around small to medium sized keratin pearls, Bar=100 µm. d: Higher magnification of Figure 4c. Yellow-brown positive expressions in the cytoplasm, Bar=50 µm. e: Poorly differentiated BOSCC. Diffuse intracytoplasmic expressions in pleomorphic tumoral areas, Bar=100 µm. f: Higher magnification of Figure 4e. Granular positive expressions in the cytoplasm, Bar=50 µm.

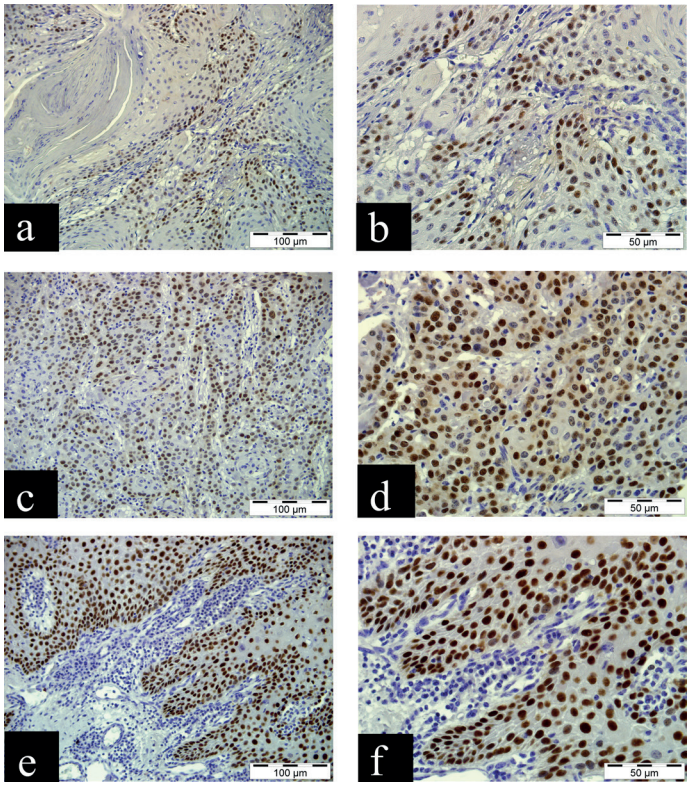

Figure 5: p53, IHC a: Well-differentiated bovine ocular squamous cell carcinoma (BOSCC). Intranuclear positive reactions in cells in the periphery of tumoral islands, Bar=100 µm. b: Higher magnification of Figure 5a. Dark brown positive expressions in the nucleus, Bar=50 µm. c: Moderately differentiated BOSCC. Immune-positive expressions in tumoral cords around small to medium sized keratin pearls, Bar=100 µm. d: Higher magnification of Figure 5c. Strong positive reactions in the nuclei of neoplastic cells, Bar=50 µm. e: Poorly differentiated BOSCC. Diffuse intranuclear expressions in atypical tumoral areas, Bar=100 µm. f: Higher magnification of Figure 5e. Strong immune-positive reactions in the nucleus, Bar=50 µm.

present in keratin pearls. In PD tumors, diffuse reaction was detected in tumoral areas dominated by parameters such as an increase in nucleolus count, abnormal mitotic figures, hyperchromasia, bizarre giant cells, and megakaryocytes (Figure 5).

Discussion

Ocular SCCs are frequently seen in cattle with unpigmented eyelids and conjunctiva (white faced cattle) such as Hereford, Holstein, and Simmental breeds. [6,8,27] Similar to the literature data, the majority of cases (90%) in our study were Simmental and Simmental cross-breed cattle. The most common age range for these tumors has been reported as 3 to 9 years by different researchers.[5,9,26] It is noteworthy that the tumor is very rare in animals younger than 3 years old and almost never occurs in animals younger than 1 year old.[27] In our study, similar to the age range reported in previous studies, the average age of the animals with the tumor was 6.1 years. No sex predisposition has been reported for this tumor8, however almost all of the cases (29 out of 30) in our study occurred in female animals. The tumor has been reported to occur more commonly in the right eye[2,5,9,26,27], but in our study, the tumor occurred more commonly in the left eye than in the right eye 16 vs 14, respectively. BOSCC has been reported to occur in several ocular and periocular regions such as the corneal junction, sclera, eyelids, third eyelid, epithelial surfaces of cornea, conjunctiva, and palpebral skin.[2,4,12,26] Similar to the results of previous studies, we observed that the tumors mostly affected the whole eye (50 %), and in the remaining cases they originated from regions such as the lateral canthus (13.33 %), lower- (16.66 %) and upper eyelids (16.66 %), and the third eyelid (3.33 %). It was not possible to determine from which ocular or periocular area the tumor originated, especially in progressive cases affecting the whole eye.

Occurrence of BOSCC has been attributed to predisposing factors such as high altitude, viruses such as papillomavi-

rus and herpesviruses, dietary habits, exposure to intense solar radiation, geographical proximity to the equator, high average temperatures, and prolonged grazing under the sun.[4,10,11,13,26] The tumor is the most common tumor observed in cattle in the Kars region. We believe that besides the predisposition of cattle breeds, high altitude of Kars and the long exposure of the cattle raised in this region to sunlight, especially in the grasslands, are effective in the formation of this tumor.

The tumor develops through two premalignant stages called epidermal plaques and papillomas, before progressing to carcinoma *in situ* and invasive carcinoma over months and years.[11,26] Carcinomas are nodular, cauliflower-like, hemorrhagic, ulcerative, friable in consistency, easily crumbly and have bad odor due to contamination.[7] In our study, we also observed that tumoral masses were cauliflower-like, nodular, hemorrhagic, ulcerative, and were covered with a purulent discharge similar to that reported in previous literature.[5,13,22,27]

The number and intensity of keratin pearls, the width of the tumoral island formation, and pleomorphism are important parameters in determining the BOSCC differentiation degrees. [2,12,26] Consistent with our results, in WD tumors, excessive and large numbers of keratin pearls, large tumoral islands, and evident squamous differentiation were reported in previous works.[6,12,26] We detected that the number and size of keratin pearls decreased in MD tumors compared to WD tumors similar to that reported by Sözmen et al.[2] In addition, we also observed that tumoral islets were smaller in these cases, similar to keratin pearls, and the number of poorly differentiated tumor cells increased similar to that reported in previous literature.[5,26] We observed that keratinization was either absent or formed in individual cells in PD tumors as reported by Azarabad et al.[22] We also found that tumor islets in PD tumors were quite small compared to MD and WD tumors, and pleomorphism increased considerably in accordance with the literature.[6,12]

PCNA is a cell cycle protein that is routinely used in tumor staging and treatment planning.1[4] Especially in canine skin and breast tumors, PCNA is an important marker used to determine the internal malignancy and growth rate.[16] In the literature review, we did not find any study in which PCNA expression was investigated immunohistochemically in SCCs of cattle. Rama Devi et al.[28] observed strong PCNA positive reaction in the nucleus of tumoral cells in a case of SCC detected in the ear of an Indian water buffalo. We also detected PCNA immune-positive reaction, especially in the nuclei of tumor cells as reported by Azarabad et al.[22] Martano et al.[15] reported that in grade 1 and grade 2 canine oral SCCs, moderate nuclear staining is especially concentrated in the peripheral part of the tumoral islands. In addition, they noted that the reaction was more diffuse and strong in grade 3 oral SCCs. Mestrinho et al.[16] and Martano et al.[15] think that PCNA expression increases in correlation with the grade of the tumor and may be an important prognostic factor especially for canine oral SCCs. We observed that the intensity of the PCNA reaction in the nuclei of tumoral cells located in the periphery of the tumoral islands surrounding the keratin pearls was quite strong in WD and MD tumors as previously reported.[14-16] In present study, we detected that PCNA positive cells were increased in PD tumors.[14-16] Contrary to the literature data, although there was a negative correlation between PCNA expression and the degree of differentiation in our study, the increase in the number of positive cells among the groups was not statistically significant. According to the data of our study, we concluded that the reliability of PCNA is controversial as a result of statistical analyzes in determining the degree of differentiation of BOSCC.

MMP-9 (also known as gelatinase b), one of the most important members of gelatinases and weighs 92-kDa, is involved in cancer initiation, development, metastasis, and progression.[29] Similar to PCNA, there are no literature data that evaluate MMP-9 expression in BOSCCs by immunohistochemical methods. Di Girolamo et al.18 reported that MMP-9 was overexpressed in human conjunctival squamous cell carcinoma compared to adjacent uninvolved conjunctival and corneal tissue from the tumor margins. Iovieno et al.[19] detected the overexpression of MMP-9 in conjunctival SCC by immunohistochemistry. In a different study, Zhang et al.[30] observed that MMP-9 is expressed and strongly involved in SCC progression and tumor invasion in mice. Mahale et al.[17] observed that MMP-9 mRNA levels increased significantly in human conjunctival SCCs. BOSCC is an invasive and chronically progressive tumor that can metastasize to the parotid lymph node, as well as invade the bone of orbit or adjacent structures.[6,11] In our study, we did not detect metastases in the parotid lymph node or any distant tissue. In addition, we demonstrated that MMP-9 expression increased in WD, MD, and PD groups compared to the control group. Although there was a negative correlation between positive cell numbers and the degree of differentiation of the tumor, we could not detect a statistically significant difference between positive cell numbers. These results made us think that MMP-9 expression should be evaluated in detail with other MMPs and different analyzes (mRNA analysis, etc.) to determine the metastatic capacity of BOSCC.

p53 is induced in response to DNA damage and acts as a transcription factor of proteins that control the cell cycle, leading to cell cycle arrest or apoptosis, particularly in the G1 phase.[12,25] It has been determined that in conjunctival

SCCs of domestic animals, especially UV initiates tumorigenesis by inducing mutations in the p53 tumor suppressor gene.[9,12,22] Cells containing mutant p53 or lacking p53 cannot arrest the cell cycle; therefore, they do not pause in G1, but continue uncontrolled into S phase before DNA repair is complete. They accumulate mutations at an increasing rate, leading to rapid selection of neoplastic clones and tumor growth.[23] We observed that p53 positive expressions were especially strong in the neoplastic cells located in the periphery of tumor islands as reported by Fornazari et al.9 Diffuse reaction was detected in pleomorphic areas in accordance with the literature.[12,22] Carvalho et al.[12] reported that no correlation between the percentage of p53 stained nuclei and the degree of differentiation was observed, although different patterns of staining were seen according to the degree of keratinization of the tumor cells. In a similar study Azarabad et al. [22] found a significant correlation between the percentage of p53-stained nuclei and the degree of differentiation. In the current study, a negative correlation was found between p53 immunopositivity and tumor differentiation.

In conclusion, we suggest that there may be a negative correlation between the differentiation degrees of BOSCCs and the increase in p53 expressions. We concluded that p53 is a very useful marker in determining the differentiation of BOSCCs.

Conflict of Interest

The authors declare that they have no conflict of interest.

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