Open Access

Check for updates

Short Communication

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.1.196-199

Pathology and evaluation of immunohistochemical expression of tumour protein 63 (p63) in corneal squamous cell carcinoma in a dog#

K.A. Abin[1], R. Anoopraj[1*], K.S. Prasanna[1], K. Krithiga[2], M.P. Laiju[3] and S. Sooryadas[4]

[1]Department of Veterinary Pathology, [3]Department of Veterinary Surgery and Radiology College of Veterinary and Animal Sciences, Mannuthy, Thrissur, [2]Department of Veterinary Pathology, Department of Veterinary Surgery and Radiology College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

Citation: Abin K.A, Anoopraj R., Krithiga. K, Laiju. M. Philip and Sooryadas S. 2024. Pathology and evaluation of immunohistochemical expression of tumour protein 63 in canine corneal squamous cell carcinoma in a dog. *J. Vet. Anim. Sci.* **56** (1):196-199

Received: 01.10.2024 Accepted: 21.12.2024 Published: 31.03.2025

Abstract

In the present study, an exophytic mass on the corneal surface in a six-year-old male Rottweiler is described. Histopathologically, neoplastic cells appeared round to polygonal with vesicular to hyperchromatic nuclei and scanty to moderate eosinophilic cytoplasm. There was infiltration of neoplastic cells into underlying stroma as well as the ulcerated fibrovascular tissue. Based on the histopathological findings, the tumor was identified as corneal squamous cell carcinoma. Immunohistochemical expression of tumour protein 63 (p63) was evaluated in the tumour and a strong expression was detected. Squamous cell carcinoma should always be a differential whenever an ulcerated mass is found in the ocular and adnexal surface[1]

Keywords: *Pathology, corneal squamous cell carcinoma, Rottweiler, tumour protein 63*

Squamous cell carcinoma is one of the common neoplasms identified in dogs. Begum *et al*. (2023) described squamous cell carcinoma as a malignant tumour of stratified epithelium and is a common neoplasm of domestic animals. Dog neoplasms are twice as common as human neoplasms, grow rapidly and share many anatomical and physiological characteristics, making canines a good animal model for better study for human malignancies (Hahn *et al.,* 1994). Takiyama *et al*. (2010) explained the incidence of primary corneal squamous cell are rare and are reported less. Bezerra *et al*. (2020) explained that trauma, loss of pigmentation in periocular tissue, chronic irritation of ocular adnexa and exposure of ultraviolet radiation could be identified as common etiological agents involved in the formation of squamous cell carcinoma. Squamous cell carcinoma is usually found in different regions of the body lined by the epithelium involving skin, ocular adnexa, lungs, penis, vagina and footpad. Chandrashekaraiah *et al*. (2011), in their study, found that dogs with squamous cell carcinoma were most commonly between the ages of six and eight, with the age range of two to eight years showing a notable predisposition. Diagnosis of cancer which is required for better treatment and prognosis, requires microscopic evaluation and immunohistochemical confirmation. Tumor protein 63 (p63), a transcription factor of p53 family, is involved in differentiation of different tissues including squamous epithelium and significantly expressed in squamous cell carcinoma (Steurer *et al*., 2021).

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

**Corresponding author: anoopraj@kvasu.ac.in, Ph. 9495361872*

A six-year-old Rottweiler was referred to the Teaching Veterinary Clinical Complex, Mannuthy with a pink, exophytic, ulcerated mass arising from the cornea of the left eye which had been progressively enlarging over the past five months with the clinical signs of conjunctivitis and epiphora. Initial ophthalmologic examination revealed normal dazzle and menace reflex. The mass was firmly attached to the cornea and superficial keratectomy was carried out.

The excised mass was preserved in 10 per cent neutral buffered formalin for histopathological studies. Formalin-fixed, paraffin-embedded tissue samples of thickness of four micrometre were subjected to haematoxylin and eosin staining (Suvarna *et al*., 2019).

Immunohistochemical analysis for p63 expression was performed on formalin-fixed, paraffin-embedded tissue samples of thickness four micrometre. The sections were deparaffinised in xylene and rehydrated in descending grades of isopropyl alcohol. Heat induced antigen retrieval method was done for p63 staining at 95-99 ^{0}C in citrate buffer (pH 6.0) for 20 minutes. Endogenous peroxide activity was blocked by incubating the sections with 3% hydrogen peroxide for 20 minutes. Bovine serum albumin was used as protein blocking agent and the sections were incubated for 20 minutes to avoid non-specific binding of antibodies. The sections were tested with rabbit polyclonal anti-p63 antibody (1:50, Elabscience Biotechnology, USA) and was incubated overnight. The sections were later treated with streptavidin-biotin-peroxidase kit (Millipore, Merck, USA) for 20 minutes. After incubation, the reaction product was detected using diaminobenzidine. The sections were later counterstained with Mayer's haematoxylin and was mounted on aqueous mounting medium. Immunohistochemical scoring was done as per Woo *et al.* (2001) and Brustmann *et al.* (2003)

Grossly, the mass was solitary, pink and firm near to lateral canthus of the left ocular corneal surface (Fig. 1). Microscopic examination revealed the neoplastic squamous cells of the corneal epithelium invading both the overlying fibrovascular mass as well as the underlying corneal stroma (Fig. 2). Corneal epithelium demonstrated hyperplasia accompanied by marked dysplasia. These dysplastic changes were characterised by haphazard arrangement of epithelial cells (Fig. 3), loss of polarity and the presence of mitotic figures, indicating high cellular

Fig. 1. Solitary, pink, exophytic mass arising from cornea (arrow)

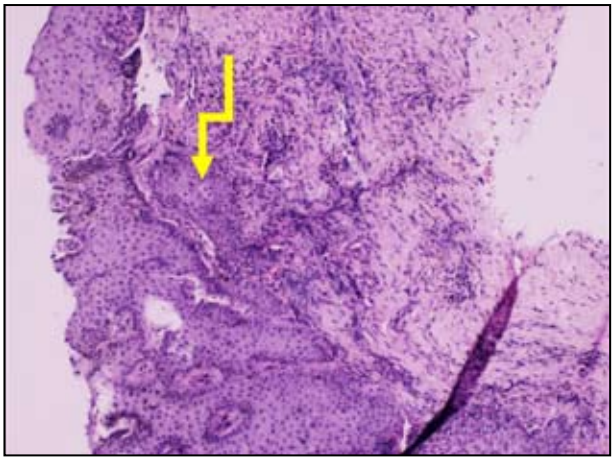

Fig. 2. Proliferating neoplastic squamous cells infiltrating into the stroma (arrow) (H&E, X100)

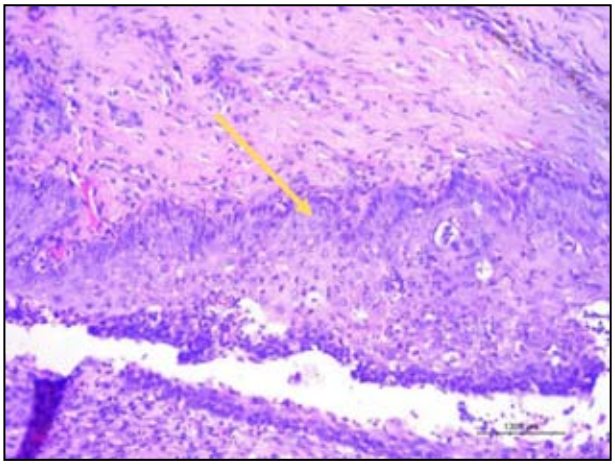

Fig. 3. Haphazard arrangement of epithelial cells (arrow) (H&E, X200)

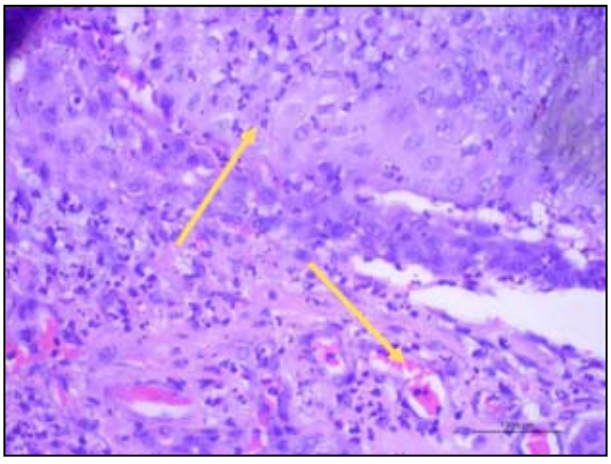

Fig. 4. Areas of ulceration with intense neutrophilic infiltration and marked vascular changes (arrow) (H&E, X400)

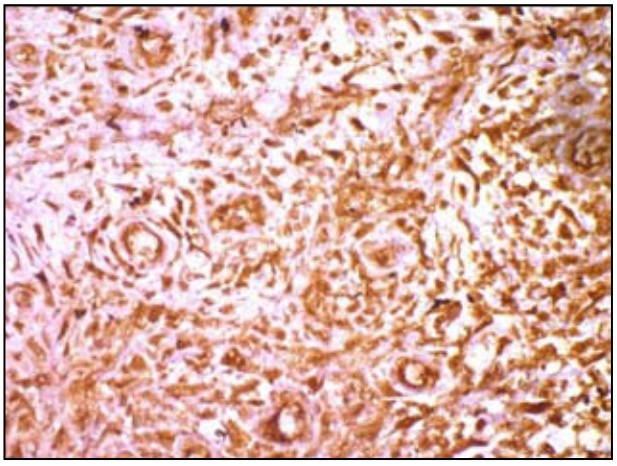

Fig. 5. Squamous cell carcinoma-p63 expression –Strong (DAB, X400)

turnover and abnormal growth patterns. The normal architecture of the corneal stroma was lost at multiple foci due to the infiltration of neoplastic cells and associated tissue reaction. The neoplastic cells appeared round to polygonal with vesicular to hyperchromatic nuclei and scanty to moderate eosinophilic cytoplasm. They showed single to multiple nucleoli. Neovascularisation was observed within the exophytic mass, suggesting the formation of new blood vessels in response to the tumour growth (Fig. 4). The neoplastic cells within the mass displayed moderate anisocytosis and anisokaryosis, with notable cellular pleomorphism and nuclear atypia. Moreover, the absence of keratinisation and presence of cell nests in the stroma indicated that the case under consideration was a squamous cell carcinoma with moderate differentiation. Inflammatory cells were abundant in the exophytic mass, reflecting an ongoing inflammatory response to the neoplastic process. The infiltration of neoplastic epithelial cells into both the corneal stroma and the exophytic mass confirms the diagnosis of invasive squamous cell carcinoma. In summary, the histological features were severe dysplasia, neovascularization and invasive behaviour collectively point to squamous cell carcinoma originating from the corneal epithelium.

Immunohistochemical evaluation of p63 was performed. The p63 showed a nuclear staining. Immunostaining in more than 75 per cent of the neoplastic cells with moderate to severe intensity was recorded (Fig. 5). Hence, the overall p63 expression in this case was adjudged as strong. Strong immunoreactivity of p63 in tumours proved immaturity of cell lineage, preserving the ability to multiply thereby proving the tumours invasiveness. Our findings were similar to the findings of Sinha *et al.* (2015).

Primary corneal squamous cell carcinoma in canines is rare and commonly reported primary corneal neoplasia are papilloma, melanocytoma, haemangioma and lymphoma (Bauer *et al*., 2015). Dogs presented with squamous cell carcinoma had a history of chronic keratitis, keratoconjunctivitis sicca or trauma (Dreyfus *et al*., 2011). Kallini *et al*. (2015) specified that resection of squamous cell carcinoma is one of the preferred methods of treatment. Łojszczyk *et al*. (2021) expressed that, squamous cell carcinoma was commonly found in areas of non-pigmented and hairless area which coincide with our findings.

The study revealed microscopic lesions indicative of moderately differentiated squamous cell carcinoma, with key pathological features including haphazard arrangement of dysplastic epithelial cells in the corneal epithelium and invasion of the exophytic mass and corneal stroma by the neoplastic squamous cells. Our observation resembled the observation made by Chandrashekaraiah *et al*. (2011).

Prognosis of squamous cell carcinoma involving corneal surface is good according to Labelle and Labelle (2013) since this neoplasia have low metastatic potential but early detection and treatment is considered safe as prognosis of globe can vary. Withrow and Vail (2007) explained that, squamous cell carcinoma related to ocular surface has less chance of metastasis to distant locations as the blood vessel walls are less penetrated by the tumorous cells.

Summary

This study reports on the pathology and immunohistochemical evaluation of p63 in a case of corneal squamous cell carcinoma (SCC) in a six-year-old Rottweiler. The occurrence of corneal SCC is rare in dogs, with trauma, ultraviolet radiation and chronic ocular irritation being the key etiological factors. Even though the metastatic potential of corneal SCC is low, early detection and surgical excision are highly essential, since it can spread if left untreated. In this study, a detailed gross and histopathologic description of the lesion was carried out. The absence of keratinisation and presence of pleomorphic cells suggested moderate differentiation, with inflammatory infiltrates further supporting the diagnosis of an aggressive neoplasm. Immunohistochemical analysis demonstrated strong nuclear p63 expression in more than 75 per cent of the tumour cells. This high expression was indicative of the low level of differentiation and invasive potential, aligning with findings in previous studies. Thus, the findings emphasise the utility of p63 immunostaining in veterinary pathology for diagnosing and prognosis of the ocular epithelial neoplasms.

Acknowledgements

The authors thank the Kerala Veterinary and Animal Sciences University for providing the research grant and other facilities for the conduct of this study.

Conflict of interest

The authors declare no conflict of interest.

References

Bauer, B., Leis, M.L. and Sayi, S. 2015. Primary corneal melanocytoma in a collie. *Vet. Ophthalmol*. **18**: 429-432.

Begum, S., Deka, A., Tamuli, S.M., Dutta, B. and Buragohain, M. 2023. Histopathological and cytopathological findings of canine squamous cell carcinoma. *Haryana Vet*. **62**: 162-164.

Bezerra, K.P.G., de Souza, M.D.F., Borges, O.M.M., de Lucena, R.B., Talieri, I.C. and de Souza, A.P. 2020. Multicentric squamous cell carcinoma with the involvement of the ocular annexes in a dog. *Acta Sci. Vet*. **48**: 511.

Brustmann, H., Riss, D. and Naudé, S. 2003. Galectin-3 expression in normal, hyperplastic, and neoplastic endometrial tissues. *Pathol. Res. Pract.* **199**: 151-158.

Chandrashekaraiah, G.B., Suguna Rao, S.R., Munivenkatappa, B.S. and Mathur, K.Y. 2011. Canine squamous cell carcinoma: a review of 17 cases. *Braz. J Vet. Pathol*. **4**: 79-86.

Dreyfus, J., Schobert, C.S. and Dubielzig, R.R. 2011. Superficial corneal squamous cell carcinoma occurring in dogs with chronic keratitis. *Vet. Ophthalmol*. **14**: 161-168.

Hahn, K.A., Bravo, L.I.N.A., Adams, H. and Frazier, D.L. 1994. Naturally occurring tumors in dogs as comparative models for cancer therapy research. *IV*. **8**: 133-144.

Kallini, J.R., Hamed, N. and Khachemoune, A. 2015. Squamous cell carcinoma of the skin: epidemiology, classification, management, and novel trends. *Int. J. Dermatol*. **54**: 130-140.

Labelle, A.L. and Labelle, P. 2013. Canine ocular neoplasia: a review. *Vet. Ophthalmol*. **16**: 3-14.

Łojszczyk, A., Łopuszyński, W., Szadkowski, M., Orzelski, M. and Twardowski, P. 2021. Aggressive squamous cell carcinoma of the cranium of a dog. *BMC Vet. Res*. **17**: 1-8.

Sinha, A., Chandra, S., Raj, V., Zaidi, I., Saxena, S. and Dwivedi, R. 2015. Expression of p63 in potentially malignant and malignant oral lesions. *J. Oral Biol. Craniofac. Res*. **5**: 165-172.

Steurer, S., Riemann, C., Büscheck, F., Luebke, A.M., Kluth, M., Hube-Magg, C., Hinsch, A., Höflmayer, D., Weidemann, S., Fraune, C. and Möller, K. 2021. p63 expression in human tumors and normal tissues: a tissue microarray study on 10,200 tumors. *Biomark. Res*. **9**: 1-14.

Suvarna, K., Layton, C. and Bancroft, J.D. 2019. *Bancroft's Theory and Practice of Histological Techniques*. (8th Ed.). Elsevier Health Sciences, Churchill, 557p.

Takiyama, N., Terasaki, E. and Uechi, M. 2010. Corneal squamous cell carcinoma in two dogs. *Vet. Ophthalmol*. **13**: 266-269.

Withrow, S.J. and Vail, D.M. 2007. Ocular tumors. In: Withrow & Mac Ewen's *Small animal clinical oncology.* (4th Ed.). Saunders Elsevier, St Louis, USA, 686p.

Woo, H.J., Joo, H.G., Song, S.W., Sohn, Y.S. and Chae, C. 2001. Immunohistochemical detection of galectin-3 in canine gastric carcinomas. *J. Comp. Pathol.* **124**: 216-218. ■