Indian J Anim Health (2026), 65(1) : 74-77
DOI: https://doi.org/10.36062/ijah.2026.07325

Research Article

Detection of p53 gene mutation in blood of white skinned Indian Khillar cattle suffering from ocular squamus cell carcinoma

V. Wagh[1*], G. A. Chandratre[2], V. Patil[1] and R. S. Nehte[3]

[1]Veterinary Surgeon, Government Veterinary Hospital, Department of Animal Husbandry and Dairying, Baramati - 413 102, Maharashtra, India: [2]Department of Veterinary Public Health and Epidemiology, LUVAS, Hisar - 125 001, Haryana, India: [3]Retired Professor, Department of Pathology. Bombay Veterinary College, Mumbai - 400 001, Maharashtra, India

Abstract

A study was conducted to detect p53 gene mutation in ocular squamus cell carcinoma in Khillar zebu cattle and its correlation with blood for early diagnosis and prognosis. Twenty blood samples and bovine eye growths of Khillar cattle of West India were collected. Blood samples were evaluated for the presence of p53 gene mutation using PCR SSCP and tissues samples were processed for detection of various grades of eye cancer. Presence of p53 mutation in blood and grades of cancer were further correlated to find any diagnostics. Out of twenty cases, six cases showed first grade, ten cases showed grade II and four cases showed grade III of invasive squamus cell carcinoma. PCR SSCP analysis showed abnormal migration patterns in five blood samples as compared to that of healthy cattle in polyacrylamide gels in present study, Mutation of *p53* gene was noted to the extent of 25% in blood. Among five samples, three samples were of grade II (15 %) whereas two samples were from grade III (10%) of squamous cell carcinoma. No mutation could be detected in the grade I. As the malignancy in ocular squamus cell carcinoma increased, p53 mutations became evident to a greater extent in circulating blood which has diagnostic and prognostic implications.

Keywords: Cattle, Khillar, Mutation, *p53* gene, Squamus cell carcinoma

Highlights

- White skinned Khillar cattle showed full differentiation of squamus cell carcinoma in the eye based on histopathological grading.
- Mutant *p53* gene is expressed in circulating blood when cells had undergone malignant transformation into grade II and grade III type
- As malignancy increased, mutations became evident to a greater extent in the circulating blood

INTRODUCTION

In India, bullocks are considered to be poor man's tractor, these draught animals are utilized for various purposes till they are agile, and their age is not determining factor in assessing their productivity. Therefore, some tumours are frequently reported in developing countries like India, compared to the developed countries, especially in farm animals (Prasanna et al., 2020; Pazhanivel, and Umarani 2022). Ocular squamous cell carcinoma is the most common and economically important neoplasm of the eye in cattle which is also called as "Cancer eye". It is a primary neoplasm of epithelial origin that may occur in different ocular and periocular tissues which includes the palpebral skin, epithelial surfaces of the cornea and conjunctiva, third eyelid, and limbus (Carvalho et al., 2005; Bilhastre et al., 2026). The aetiology and predisposition of this cancer is multifactorial, includes genetic susceptibility, circumocular pigmentation, age, and ultraviolet radiation (Tsujita et al., 2010; Sugan et al., 2024). Continuous exposure to ultra-violet (UV) radiations is one of the important predisposing factor in non-pigmented animals. These radiations cause DNA damage resulting in mutation of oncogenes and tumor-suppressor genes resulting into neoplasia. Among various mutations, one of the genetic lesions is mutation in the tumor suppressor *p53* gene (Brash et al., 1991; Setoguchi et al., 2001) commonly observed in radiation light–induced cutaneous squamous cell carcinomas. Cattle with non-pigmented eyelids and conjunctiva (white faced cattle) show a greater predisposition towards development of UV induced eye cancer (Anderson & Badzioch,1991). In India, white skinned Khillar breed of cattle, one of the best draught breed is predisposed to squamus cell carcinoma

*Corresponding author, E-mail: drvirajpath1@gmail.com

of eye due to lack of circum-ocular pigment. Prolonged exposure to sunlight (UV rays) is accounted to be a driving force for this neoplastic condition. It has been reported that ultraviolet rays of the sun are reflected onto the skin around the eye in white faced cattle which results in damage to the DNA of the tissue and lead to cancer eye. Similar observations has also been reported in Hereford cattle which lack the circumocular pigment (Sugan et al., 2024). In this study it has been hypothesized that by evaluating the mutational status of *p53* gene in circulating DNA from the blood of Khillar cattle affected with eye cancer can be useful marker in early diagnosis and assessing the therapeutic efficacy of cancer. Further there are no reports on molecular detection of p53 gene mutation in blood of eye cancer affected Khillar cattle. Thus the aim of present study is to assess the status of p53 gene mutation in blood samples of Khillar cattle.

MATERIALS AND METHODS

A study was conducted on 20 Khillar cattle having eye growths in and near the eye. The blood samples and tissue samples from the affected animals and six blood samples from apparently healthy cattle (six samples) were collected from the Deonar slaughter house, Mumbai, were preserved and transported on ice in anticoagulant containing vial for PCR-SSCP and stored at -20°C for further downstream analysis. Since samples were collected from the dead animals at abattoir, prior animal ethics committee permission was not required. Blood samples of apparently healthy cattle were analyzed for hematological and biochemical alteration if any using automated VETSCAM HM5 counter and ERBA Chem 5x respectively to categorize them under healthy cattle. Tissue samples were preserved in 10% formalin for histopathological examination and processed by paraffin wax embedding method (Luna 1968). Sections were cut at 6-7 µm thickness and stained with haematoxylin and eosin (H&E) stain. The slides were observed under light microscope for histopathological characterization of tumors.

Total DNA from 20 blood samples of affected animals and 6 apparently healthy animals were extracted from the circulating whole blood using commercially available kit (Quigen, India) as per manufacturer's instruction. The quality and quantity of extracted DNA was evaluated. DNA concentration was standardized to 30 ng/µL and subjected to PCR for amplification of the *p53* tumor suppressor gene using two primers SP 1 (sense) CCTCTGAGCCAGGAGACATT and SP 2 (Antisense)AGGTCACGGACTTGCCTGTT (for different regions of *p53* gene published earlier (Chandratre et al., 2020). PCR-SSCP based detection of mutations in *p53* gene t was done on PCR product. Analysis of gel was done for band pattern determination.

RESULTS

Gross examination of eye showed presence of reddish growth in eye. (Fig. 1A). Further complete blood count (CBC) and biochemical tests (Liver and Kidney function) did not revealed any alteration indicating healthy status of animal. H & E sections were examined to confirm the nature of eye lesions. Twenty representative sections were confirmed as eye cancers which were sub-divided into three grades based on various criteria as depicted in Table 1. Out of twenty cases, six animals showed first grade invasive squamous

Table 1. Basic criteria for histopathological grading of ocular squamus cell carcinoma

Criteria	Grade I	Grade II	Grade III
Epithelial pearls	Small in size, comprising of 2-3 layers of epithelial cells with scanty keratin	Well developed epithelial pearls of large size and irregular shape	No pearls; Extensive proliferation of squamous cells which are arranged in homogenous sheets of neoplastic cells
Mitotic figures	6 to 10/hpf[#]; Present in c ells around the keratin	30-50/hpf; Present in proliferating cells	60-80/hpf in anaplastic cells
Extent of anaplasia	Minimal extent	Moderate extent	Highly anaplastic cells with large hyperchromatic nucleus and prominent nucleoli
Pleomorphism	minimal pleomorphism	Proliferating cells were arranged as islands or clusters;Mild pleomorphism	Severe pleomporphism in cells
Presence of inflammatory cells	+	++	+++

#hpf: High power field; +++: large number, ++: moderate number, +: few number

cell carcinoma (Fig. 1B), i.e., 30% of total SCC. Ten cases were classified under grade II forming 50% of total SCC (Fig. 1C); whereas, four cases were of grade III, i.e., 20% of the total SCC (Fig. 1D). Upon PCR amplification a 550 bp product was obtained from DNA targeted of all the blood samples of affected as well as healthy animals (Fig. 2A). SSCP analysis of the PCR amplified product of *p53* tumor suppressor gene was done to detect any possible nucleotide sequence variation among blood samples. Mutation associated SSCP band shifts were identified considering difference in migration pattern of SSCP bands. SSCP analysis showed abnormal migration patterns in five blood samples as compared to that of healthy cattle in polyacrylamide gels (Fig. 2B). Thus in present study, mutation of *p53* gene was noted to the extent of 25% in blood. Among five samples, three samples were of grade II of squamous cell carcinoma (15%) whereas two samples were from grade III (10%). No mutation could be identified in the grade I but as the malignancy increased, mutations became evident to a greater extent in circulating blood as detected by PCR-SSCP.

DISCUSSION

In this study, authors tried to correlate the extent of malignancy with occurrence of p53 gene mutation in the blood to understand its diagnostic use. Typical histological findings such as formation of epithelial pearls and cell nests with anaplastic cells having mitotic figures in grading of bovine SCC of eye (Ceylan et al., 2012; Karakrut et al., 2021) have been reported by researchers. They further noted extensive deposition of concentric layers of keratin and epithelial pearls, which contained few proliferating cells at the periphery when growth was at distal regions (Fornazari et al., 2017). Over expression of *p53* gene by immune histochemistry in ocular SCC tissue has been reported by earlier workers (Karakrut et al., 2021; Kumar et al., 2024). Wherein p53 expression in cattle SCC characterized by poor histopathological grading, suggested an essential role of *p53* in diagnosis of moderately and poorly differentiated SCC.

In the present study, authors also tried to correlate between grade of squamus cell carcinoma diagnosed histopathologically and either presence or absence of mutations in the blood. Mutations at the rate of 25% observed in the present study suggested that mutations in the *p53* gene might invariably be involved in various neoplastic conditions which could be evident in the circulating blood besides neoplastic tissue. Mutation detected in grade II and grade III of eye cancer suggest that mutant *p53* is expressed in circulating blood when tumor cells undergo malignant type transformation which is very significant finding. Similarly, study done in canine lymphoid neoplasia showed presence of higher concentration of plasma DNA which correlated with higher tumour burden and poor outcome in dogs (Schaefer et al., 2007).

Further in present study, presence of eye tumors in white skinned Khillar breed supports the hypothesis that there is a role and association of solar radiation and depigmentation in formation of ocular squamus cell carcinoma (Kumar et al., 2024). Bovine OSCC prevalence has already been directly related to exposure to UV radiation and, as with humans, it seems highly probable that a mutation in the gene that encodes for this protein is, at least, one of the factors involved in the genesis of this tumor.

As the malignancy in ocular squamus cell carcinoma increased, p53 mutations became evident to a greater extent in circulating blood which has diagnostic and prognostic implications and needs further study. The study of mutations in the *p53* gene along with sequencing to locate position of mutation in blood of animals having bovine ocular squamus cell carcinoma may provide useful information regarding the diagnosis, prognosis and treatment of eye cancer in cattle.

Conflict of interest: Authors declare that there is no conflict of interest.

Author's contribution: VW: Involved in investigation, data generation, preparing original draft; GAC: Engaged in conceptualization, data curation, supervision and final editing; VP: Involved in methodology and editing; RSN: Histopathology photography and final check

Data availability statement: Data set generated during research will be available from the corresponding author upon reasonable request

Use of artificial intelligence tools: During the preparation of this work, the authors have not used any artificial intelligence tool.

REFERENCES

Anderson, D. E., & Badzioch, M. (1991). Association between solar radiation and ocular squamous cell carcinoma in cattle. *American Journal of Veterinary Research*, *52*(5), 784–788.

Bilhastre, B., Vala, H., Ribeiro, C. A., Faria, S., Oliveira, A., Branco, S., & Pinto, C. (2026). Bovine ocular squamous cell carcinoma—A descriptive epidemiological survey in the Azores, Portugal. *Veterinary Sciences*, *13*(4), 371. https://doi.org/10.3390/vetsci13040371

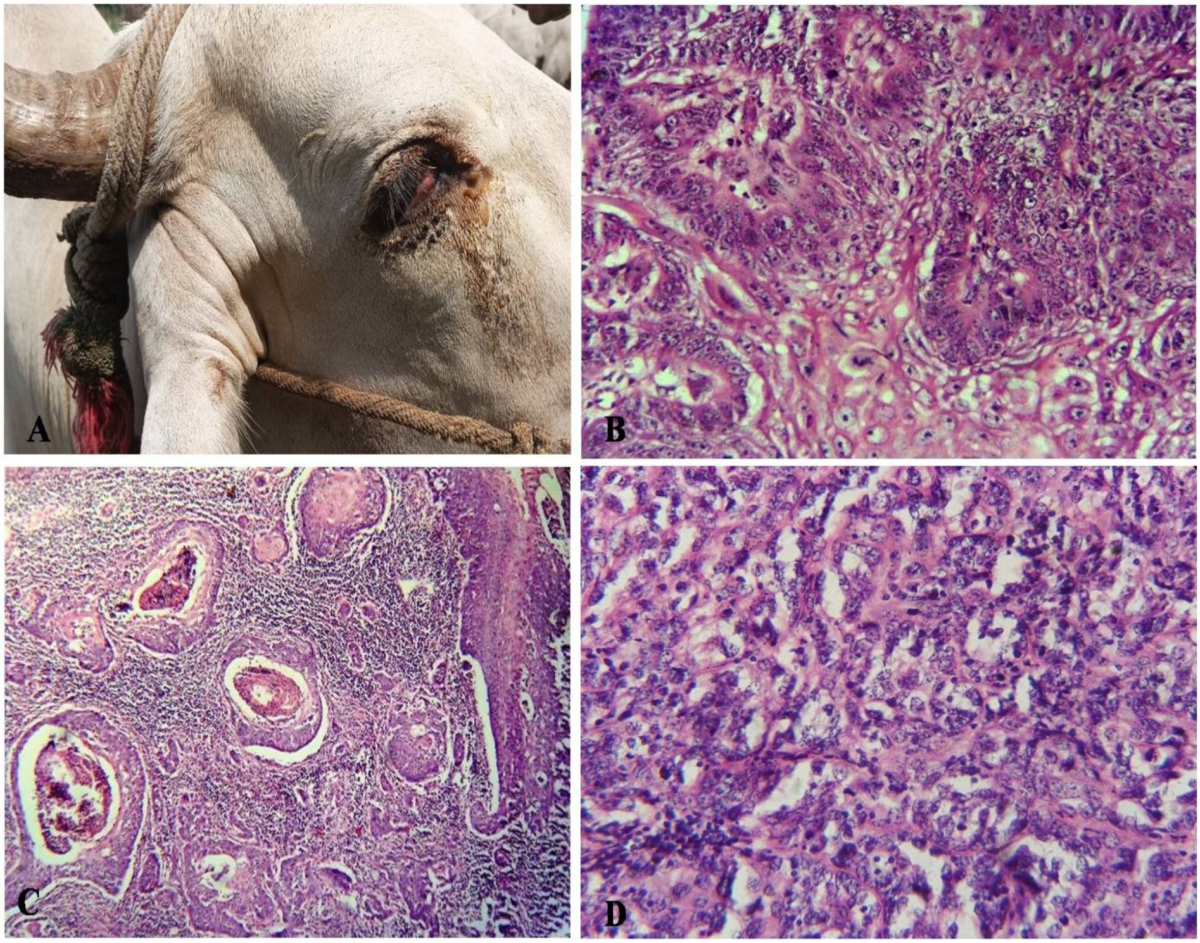


Fig. 1. Ocular squamus cell carcinoma (SCC) in Khillar cattle. A: Reddish growth of SCC on the inner canthus of eye, B : Neoplastic cells forming nest and separated by fibrous tissue proliferation in grade I of SCC, C: Well developed nests of neoplastic cells with keratin pearls inside and heavy infiltration of inflammatory cells in grade II of SCC, D: Haphazard arrangement of neoplastic cells and abundant mitotic figures in grade III of SCC

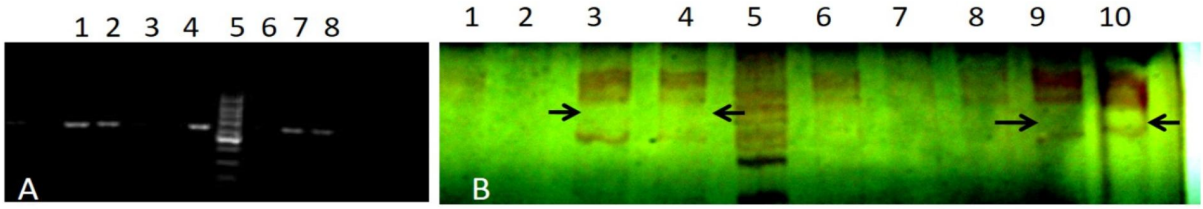


Fig. 2A. PCR amplification of bovine p53 gene segment. Lane 1, from animal with grade I neoplasm. Lane 2, from animal with grade II neoplasm. Lane 3, negative control (no DNA template. Lane 4, from animal with grade III neoplasm. Lane 5, 100bp DNA ladder. Lane 6, negative control , Lane 7 and 8 control animal. A product of 550 base pairs is present in lanes 1, 2, 4, 7 and 8 but absent in lanes 3 and 6

Fig. 2B. SSCP of amplicons from plasma DNA of Khillar cattle with eye cancer and healthy control cattle. Lane 1, animal with grade I neoplasm. Lane 2, negative control animal. Lane 3, animal with grade II neoplasm. Lane 4, animal with grade III neoplasm. Lane 5, 100bp DNA ladder. Lane 6, healthy control animal. Lanes 7 and 8, animals with grade I neoplasm. Lane 9, animal with grade II neoplasm. Lane 10, animal with grade III neoplasm. Note differences in DNA migration patterns (arrow) in samples four grade II and III cancer cases (lanes 3, 4, 9 and 10) compared with the healthy cattle (lanes 6) and cattle with grade I neoplasms (lanes 1, 7 and 8)

Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J. Baden, H. P., Halperin, A. J., & Pontén, J. (1991). A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proceedings of the National Academy of Sciences*, *88*(22), 10124–10128. https://doi.org/10.1073/pnas.88.22.10124

Carvalho, T. H., Vala, C., Pinto, 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. https://doi.org/10.1354/vp.42-1-66

Ceylan, C., Ozyildiz, Z., Yilmaz, R., & Biricik, H. S. (2012). Clinical and histopathological evaluation of bovine ocular and periocular neoplasms in 15 cases in Sanliurfa region. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, *18*(3), 469–474. https://doi.org/10.9775/kvfd.2011.5710

Chandratre, G. A., Wagh, V., Badgujar, P. C., Patil, V., & Nehte, R. S. (2020). p53 gene mutation in blood of Zebu cattle with squamous cell carcinoma of the horn. *Journal of Comparative Pathology*, *181*, 53–57. https://doi.org/10.1016/j.jcpa.2020.09.018

Fornazari, G. A., Kravetz, J., Kiupel, M., Sledge, D., Filho, I. R. B., & Montiani-Ferreira, F. (2017). Ocular squamous cell carcinoma in Holstein cows from the South of Brazil. *Veterinary World*, *10*(12), 1413–1420. https://doi.org/10.14202/vetworld.2017.1413-1420

Karakurt, E., Uður, A., Enver, B., Serpil, D., Celal, Þ. E., Özgür, A., Hilmi, N., Ayfer, Y., & Kurtbaþ, E. (2021). Expression of PCNA, MMP-9 and P53 in bovine ocular squamous cell carcinomas: An immunohistochemical study. *Journal of Research in Veterinary Medicine*, *40*(2), 98–105. https://doi.org/10.30782/jrvm.937240

Kumar, V., Jolhe, D. K., & Ghosh, C. R. (2024). Review on pathological and diagnostic features of horn and ocular squamous cell carcinoma in ruminants. *International Journal of Veterinary Sciences and Animal Husbandry*, *9*(6), 499–502.

Luna, L. G. (1968). *Manual of histologic staining methods of the Armed Forces Institute of Pathology* (3rd ed.). McGraw-Hill Book Co.

Pazhanivel, N., & Umarani, R. (2022). Current status of bovine ocular squamous cell carcinoma. *Indian Journal of Veterinary Pathology*, *46*(3), 183–192.

Prasanna, L. M. P., Veena, R. V., Suresh Kumar, D., Rani, P. K., Reddy, J. M., & Veda, S. J. (2020). Management of bovine eye cancer with autogenous vaccine. *International Journal of Current Microbiology and Applied Sciences*, *9*(5), 2734-2737. https://doi.org/10.20546/ijcmas.2020.905.313

Schaefer, D. M. W., Forman, M. A., Kisseberth, W. C., Lehman, A. M., Kelbick, N. T., Harper, P., & Lush, L. J. (2007). Quantification of plasma DNA as a prognostic indicator in canine lymphoid neoplasia. *Veterinary and Comparative Oncology*, *5*(3), 145–155. https://doi.org/10.1111/j.1476-5829.2007.00122.x

Setoguchi, A., Sakai, T., Okuda, M., Minehata, K., Yazawa, M., Ishizaka, T., Watari, T., Nishimura, R., Sasaki, N., Hasegawa, A., & Tsujimoto, H. (2001). Aberrations of the p53 tumor suppressor gene in various tumors in dogs. *American Journal of Veterinary Research*, *62*(3), 433-439. https://doi.org/10.2460/ajvr.2001.62.433

Sugan, G., Velavan, A., Vijayakumar, M., Krupakaran, R. P., Kathirvel, S., Gopal, K., Dharmaceelan, S., & Arivubalagan, S. (2024). Incidence of ocular squamous cell carcinoma in cows: A retrospective study. *International Journal of Veterinary Sciences and Animal Husbandry*, *9*(5), 232–236.

Tsujita, H., & Plummer, C. E. (2010). Bovine ocular squamous cell carcinoma. *Veterinary Clinics of North America: Food Animal Practice*, *26*(3), 511–529. https://doi.org/10.1016/j.cvfa.2010.08.003

Received - 10.05.2025, *Accepted - 03.05.2026*, *Published - 10.05.2026 (Online), 01.06.2026 (Print)*
Section Editor: Dr. Chandra Kanta Jana, Editorial Board Member