Case Report / Olgu Sunumu

Clinicopathologic evaluation of oral squamous cell carcinoma in a young dog

Hazal ÖZTÜRK GÜRGEN1,a, Evrim EGEDEN2,b, Gülbin ŞENNAZLI1,c

1İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, Department of Pathology, İstanbul; 2Ada Veterinary Policlinic, İstanbul, Turkey.

Receipted date: 17.12.2019- Accepted date: 18.04.2020

Abstract: Canine oral papilloma is a benign tumor of young dogs and caused by papillomavirus. The possible role of papillomavirus infection in the development of oral squamous cell carcinoma has recently been studied, but it has not been elucidated in veterinary medicine yet. A one-year-old, mixed, spayed, female dog was presented with severely disseminated oral lesions, lethargy, and weight loss. Physical examination of the patient revealed severely disseminated oral papillomatous lesions in the entire oral cavity and the complete blood test showed mild non-regenerative anemia and pancytopenia. In addition, the patient was found seropositive by the SNAP 4Dx Plus test for Ehrlichia canis. Histopathologic examination of oral lesions was performed using Hematoxylin and Eosin (HE) staining and immunohistochemistry for p16 antibody which increases in infections caused by papillomavirus. Histopathology revealed the histologic features of oral papilloma in association with squamous cell carcinoma. Cytoplasmic and nuclear positive reactions for p16 protein were observed within the neoplastic cells in the immunohistochemical examination. Thereafter, the dog was treated with combined therapy of vincristine, antibiotic, radiotherapy, and high doses of vitamin C. After long-term treatment, the dog completely recovered from the lesions. In this report, it was aimed to present a possible role of papilloma in the development of oral squamous cell carcinoma with the clinical, histopathological, immunohistochemical findings and treatment procedure.

Keywords: Canine, immunohistochemistry, oral papilloma, squamous cell carcinoma, treatment

Genç bir köpekte oral skuamöz hücreli karsinomun klinikopatolojik değerlendirme

Özet: Oral papilloma, genç köpeklerin iyi huylu bir tümörüdür ve papillomavirüs enfeksiyonundan kaynaklanır. Papillomavirüsün oral skuamöz hücreli karsinomu gelişimindeki olağan rolünü son yıllarda belirgine getirilmiş, fakat Veteriner Hekimlikte bu henüz ortaya konulmamıştır. Bir yaşındaki, melez, kısırlaştırılmış, dişi köpek, şiddetli dağılım gösteren oral lezyonlar, letarji ve kilo kaybı şikayeti ile getirildi. Hastanın fiziksel muayenesinde tüm ağzı boşluğuna yayılmış şiddetli papillomatöz lezyonlar saptandı. Tam kan testinde regeneratif olmayan haffan anemi ve pansitopeni tespit edildi. Ayrıca, yapılan SNAP 4Dx Plus testi ile hasta Ehrlichia canis seropozitif bulundu. Oral lezyonların histopatolojik incelemesi Hematoksilen ve Eosin (HE) boyama yöntemi ve papillomavirüs enfeksiyonu ile ilişkili olan p16 antikor kullanılarak yapıldı. Farklı tıbbi tespitlerdeki bu informasyonlarla, papillomavirüs ve oral lezyonlarla ilişkili olan bir kanser tanısı konuldu. Ehrlichia canis ile ilişkili olan bu lezyonlarla uyumlu bulunan p16 proteinin hücreli reaksiyonları gözlemlendi. Daha sonra vincristine, antibiyotik, radyoterapi ve yüksek C vitamini dozlarının kombinasyonu uygulandı. Uzun süreli bir tedaviden sonra, hastada izlenen lezyonlar tamamen iyileşti. Bu olgudan papillomavirüs ile ilişkili oral skuamöz hücreli karsinom gelişimindeki potansiyel rolü daha da açık kılan bir durum olmuştur. Bu olgudan, papillomavirüsün oral lezyonlarla ilişkisi ve bu durumun tedavisinde rolü değerlendirilmelidir.

Anahtar sözcükler: Immunohipnotik, köpek, oral papilloma, squamöz hücreli karsinom, tedavi

Papillomaviruses (PVs) are non-enveloped, double-stranded DNA viruses with a circular genome of 8000 pairs. They are known to induce epithelial proliferation on the skin and mucous membranes in their natural hosts and related species (5). Oral papillomas are characterized by cauliflower-like exophytic warts, but can also be fringed or nodular, and arise in oral mucosa, including lips and mucocutaneous junctions (12). They are commonly seen
in young and immunosuppressed dogs (24). So, the diagnosis of PV infection in dogs is quite simple by clinical examination if the animal is young. But diagnostic methods are based on classical histopathology and polymerase chain reaction (PCR), as well as immunohistochemistry, in situ hybridization and electron microscopy (12).

Oral papilloma is accepted to be a benign tumor and usually regresses spontaneously (4). On the other hand, excessive proliferation of the epithelium can also result in malignant transformation of the lesion to squamous cell carcinoma (SCC) (3, 20, 26). Although there are several possible causes for the development of oral SCC in dogs, the actual role of PV infection remains uncertain (20).

Clinical approach to the oral SCC depends on the prognosis of the patient and includes surgical excision (19), chemotherapy (6), radiotherapy (8), photodynamic therapy (14), or combined therapy of these options (13, 22). Here, it was aimed to present the clinicopathologic evaluation of a young dog with severe oral papilloma progressing to oral SCC regarding increased attention on the possible role of PV infection in the development of oral SCC since the last decade in veterinary medicine, especially in dogs and cats.

A one-year-old, mixed breed spayed female dog was presented with the complaints of severely disseminated oral lesions, emaciation due to feeding difficulty, salivation, and mild anemia. In clinical examination, oral lesions were characterized by multiple, white to pinkish, pedunculated, cauliflower-like exophytic masses ranging from 1.5 to 3 cm in diameter and were present throughout the oral cavity including; the tongue, palatopharyngeal mucosa, superior and inferior labial mucosa, buccal mucosa, upper and lower lips and extending to the hairy skin (Figure 1). The masses located on the superior and inferior labial mucosa were also characterized by ulcerative changes. Complete blood test revealed mild non-regenerative anemia and pancytopenia. In addition, the dog was found sero-positive by SNAP 4Dx Plus test for *Ehrlichia canis*. A single biopsy sample taken from the inferior labial mucosa was fixed in 10% formalin solution, embedded in paraffin, sectioned, and stained with HE. After preliminary histopathological evaluation, the biopsy sample was stained by streptavidin-biotin immunoperoxidase method, using anti- p16\(^{NK4a}\) (E6H4), a mouse monoclonal primary antibody (REF 705-4713, ready-to-use, Ventana, Arizona) and Seconder antibody (REF 253-2188, ready-to-use, Ventana, Arizona). Immunoreaction was visualized by diaminobenzidine and the section was counterstained with Mayer’s hematoxylin.

Histopathological findings revealed characteristic features of papillomavirus infections with severe papillomatous hyperplasia within the mucosal epithelium and hyperkeratosis (Figure 2A), koilocytosis (Figure 2B), intra-nuclear eosinophilic inclusion bodies in the epithelial cells (Figure 2A, inset). Nonetheless, squamous neoplastic cells were observed to have arranged in nests or trabeculae-like structures, supported by fibrovascular stroma within the lamina propria and submucosa. The neoplastic cells, which were round-to-polygonal-shaped with eosinophilic cytoplasm, exhibited varying degrees of squamous differentiation, namely formation of keratin pearls, and also single-cell keratinization (Figure 3A). Neoplastic cells showed severe hyperchromasia, anisocytosis, anisokaryosis and atypical mitotic figures, and some neoplastic cells had multiple nuclei. In addition, infiltration of inflammatory cells and superficial ulceration were also observed (Figure 3B). Immunohistochemical staining against p16 antigen was visualized as cytoplasmic and nuclear positive reaction in a pattern of diffuse and homogenous brown coloring in the neoplastic cells (Figure 4). The case was diagnosed as severe oral papillomas progressing to oral SCC. A treatment protocol was concordantly established by the
Figure 2. A. The appearance of papilloma with severe papillomatous hyperplasia (arrowhead) within the mucosal epithelium and hyperkeratosis (star). H&E. Bar = 100 µm. B. Numerous koilocytes with a swollen nucleus surrounded by a clear halo (arrows). H&E. Bar = 20 µm. Intra-nuclear eosinophilic inclusion bodies in the epithelial cell (2A, inset). H&E. Bar = 10 µm.

Figure 3. A. High-grade epithelial hyperplasia from the mucosa to the submucosa in the canine oral cavity. Neoplastic differentiation characterized by different degrees of keratinization (horn pearls /arrow) in squamous epithelial cells clustered as nests. H&E. Bar = 100 µm. B. Superficial ulceration on the mucosa with severe infiltration of polymorph neutrophil leukocytes (star). H&E. Bar = 50 µm.

Figure 4. Positive immune reaction against P16 antigen in neoplastic canine oral SCC (arrows). IHC with Anti-p16INK4a/E6H4. Bar = 20 µm.
Figure 5. Decreased PV lesions after the treatment with vincristine and prominent ulcerative SCC in the oral cavity.

administration of vincristine (0.75 mg/kg IV, Vincristine®, Kocak Farma, Turkey) with 0.9% NaCl isotonic serum (10 ml/kg, Deva®, Turkey) once a week for 6 weeks and by doxycycline (10 mg/kg/day PO) for a month to improve the ehrlichiosis of the patient. Substantial improvement was achieved in oral PV lesions (Figure 5) by vincristine administration. Then, the patient also received 12 fractions of 48 Gy radiotherapy 3 times a week in 12 sessions. In addition, vitamin C (1 g/kg) was administered during the treatment. After the treatment, the lesions on the oral mucosa and the hairy skin completely healed.

The possible role of PV infection in the development of oral SCC has recently been studied in both human and veterinary medicine. While PV infection is considered to be one of the causes of head and neck tumors in human medicine (7), it is still questionable in veterinary medicine (17, 15, 26). In the presented case report, the gross and histopathological findings were found compatible with the oral SCC accompanied by severe oral papilloma. The young age of the dog was deemed to be the predisposing factor for PV infection, as previously indicated (25), and subsequently, the PV infection was assumed to have developed into SCC. Therefore, additional immunohistochemical staining was performed against p16, antigen which has been intended to underline a possible PV etiology in oral SCC (23). It has been shown that human PV infection has a breakdown effect on cell cycle regulation by affecting the function of retinoblastoma protein (2). It causes degradation of retinoblastoma protein, which results in the increased amount of cellular p16 protein (21). While immunodetection of p16 protein is currently in use as an indicator for the presence of PV in human medicine (11), the reports in veterinary medicine need to be improved (18, 16, 20). For instance, the role of PV in SCC has been investigated by both IHC for p16 and amplification of PV DNA via PCR in canine oral SCC, despite the positive results for p16 immunopositivity, DNA of the PV hasn’t been detected in any case of that study (16). In the present study, due to the lack of PCR analysis the existence of PV infection and coexistence of oral SCC was diagnosed based on the typical macroscopic and histologic features of the animal. Although the immunohistochemical findings revealed the potential role of PV infection-induced development of SCC, the actual role of PV remains uncertain.

The dog was initially treated with vincristine for PV infection. Concurrent therapy was administered by vitamin C and doxycycline, which is a tetracycline group of antibiotics used with adequate doses for ehrlichiosis (9). Following the regression of the viral papillomas and the improvement of the immunity, the dog received radiotherapy for oral SCC. Radiotherapy has already been recommended as a sole treatment method or an adjuvant treatment for incompletely excised oropharyngeal SCC in dogs (19). In humans, concurrent chemotherapy and radiotherapy have widely been accepted for the management of locally advanced epithelial tumors (10) and have been established as standard non-surgical therapy for patients with head and neck carcinomas (1).

In conclusion, this case can provide additional support for the possible role of papillomavirus in the development of oral SCC, which was supported by macroscopic, histopathologic, and immunohistochemical findings. In addition, the treatment of choice was proved quite effective and the dog completely recovered after the treatment. Combination of vincristine, antibiotics, and vitamin C exhibited a favorable outcome in the regression of oral papilloma. The treatment protocol was completed with radiotherapy for oral SCC. The condition of the dog is known to have remained stable since the termination of the treatment. Therefore, this case was considered to be a contribution to veterinary literature.

Financial Support

This research received no grant from any funding agency/sector.
Ethical Statement
This study does not present any ethical concerns.

Conflict of Interest
The authors declared that there is no conflict of interest.

References
1. Argiris A (2002): Update on chemoradiotherapy for head and neck cancer. Curr Opin Oncol 14, 323-329.
2. Boyer SN, Wazer DE, Band V (1996): E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. Cancer Res, 56, 4620-4624.
3. Bregman CL, Hirth RS, Sundberg JP, et al (1987): Cutaneous neoplasms associated with canine oral papilloma virus vaccine. Vet Pathol 24, 477-487.
4. Chambers VC, Evans CA (1959): Canine oral papillomatosis I. Virus assay and observations on the various stages of the experimental infection. Cancer Res, 19, 1188-1195.
5. De Villiers EM, Fauquet C, Broker TR, et al (2004): Classification of papillomaviruses. Virology 324, 17-27.
6. De Vos JP, Burm AGD, Focker AF, et al (2005): Piroxicam and carboplatin as a combination treatment of canine oral non-tonsillar squamous cell carcinoma: a pilot study and a literature review of a canine model of human head and neck squamous cell carcinoma. Vet Comp Oncol, 3,16-24.
7. Gillison ML (2007): Current topics in the epidemiology of oral cavity and oropharyngeal cancers. Head Neck, 29, 779-792.
8. Grier CK, Mayer MN (2007). Radiation therapy of canine nontonsillar squamous cell carcinoma. Can Vet J, 48,1189-1191.
9. Harrus S, Kenny M, Miara L, et al (2004). Comparison of simultaneous splenic sample pcr with blood sample pcr for diagnosis and treatment of experimental ehrlichia canis infection. Antimicrob Agents Chemother, 48, 4488-4490.
10. Hennequin C, Favaudon V (2002): Biological basis for chemo-radiotherapy interactions. Eur J Cancer, 38, 230-233.
11. Konig F, Krekeler G, Höng JC, et al (2007). Relation between human papillomavirus positivity and p16 expression in head and neck carcinomas – A tissue microarray study. Anticancer Res, 27, 283-288.
12. Lange CE, Favrot C (2011). Canine papillomaviruses. Vet Clin Small Anim, 41, 1183-1195.
13. Mas A, Blackwood L, Cripps P, et al (2011). Canine tonsillar squamous cell carcinoma – a multi-centre retrospective review of 44 clinical cases. J Small Anim Pract, 52, 359-365.
14. McCaw DL, Pope ER, Payne JT, et al (2000): Treatment of canine oral squamous cell carcinomas with photodynamic therapy. Br J Cancer, 82, 1297-1299.
15. Munday JS, Dunowska M, De Grey S (2009): Detection of two different papillomaviruses within a feline cutaneous squamous cell carcinoma. N Z Vet J, 57, 248-51.
16. Munday JS, French A, Harvey CJ (2015a): Molecular and immuno-histochemical studies do not support a role for papillomaviruses in canine oral squamous cell carcinoma development. Vet J, 204, 223-225.
17. Munday JS, Kiupel M, French AF, et al (2008): Amplification of papillomaviral DNA sequences from a high proportion of feline cutaneous in situ and invasive squamous cell carcinomas using a nested polymerase chain reaction. Vet Dermatol, 19, 259-263.
18. Munday JS, Knight CG, French AF (2011). Evaluation of canine oral squamous cell carcinomas for p16CDKN2A protein immunoreactivity and the presence of papillomaviral DNA. Res Vet Sci, 90, 280-283.
19. Munday JS, Löhr CV, Kiupel M (2017): Tumors of the alimentary tract. 500-507. In: Meuten DJ (ed.): Tumors in Domestic Animals. John Wiley & Sons, Inc. Ames, Iowa.
20. Munday JS, Tucker RS, Kiupel M, et al (2015b): Multiple oral carcinomas associated with novel papillomavirus in a dog. J Vet Diagn Invest, 27, 221-225.
21. Parry D, Bates S, Mann DJ, et al (1995): Lack of cyclin D-Cdk complexes in Rb-negative cells correlates with high levels of p16INK4A/MTS1 tumor suppressor gene product. EMBO J, 14, 503-511.
22. Rejec A, Benoit J, Tutt C, et al (2015): Evaluation of an accelerated chemoradiotherapy protocol for oropharyngeal squamous cell carcinoma in 5 cats and 3 dogs. J Vet Dent, 4, 212-221.
23. Smeets SJ, Hesselink AT, Spell EJM, et al (2007): A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer, 121, 2465-2472.
24. Sunberg JP, Smith EK, Herron AJ, et al (1994): Involvement of canine oral papillomavirus in generalized oral and cutaneous verrucosis in a chinese shar pei dog. Vet Pathol, 31, 183-187.
25. Sykes J, Luff JA (2014): Viral papillomatosis. 261-268. In: Skyes JE (ed.), Canine and Feline Infectious Diseases. Saunders, Elsevier, Missouri.
26. Teifke JP, Löhr CV, Shirasawa H (1998): Detection of canine oral papillomavirus-DNA in canine oral squamous cell carcinomas and p53 overexpressing skin papillomas of the dog using the polymerase chain reaction and non-radioactive in situ hybridization. Vet Microbiol, 60, 119-130.