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Case Report / *Olgu Sunumu*

Clinicopathologic evaluation of oral squamous cell carcinoma in a young dog

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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 değerlendirilmesi

Özet: Oral papilloma, genç köpeklerin iyi huylu bir tümörüdür ve papillomavirüs enfeksiyonundan kaynaklanır. Papillomavirüsün oral skuamöz hücre karsinomu gelişimindeki olası rolü son yıllarda çalışılmıştır, fakat veteriner hekimlikte bu henüz ortaya konulmamıştır. Bir yaşında, melez, kısırlaştırılmış, dişi köpek şiddetli dağılım gösteren oral lezyonlar, letarji ve kilo kaybı şikâyeti ile getirildi. Hastanın fiziksel muayenesinde tüm ağız boşluğuna yayılmış şiddetli papillomatöz lezyonlar saptandı. Tam kan testinde rejeneratif olmayan hafif 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 papillomaviral enfeksiyonlarda artış gösteren p16 antikoru ile immünohistokimyasal olarak yapıldı. Mikroskobik bakıda, vakaya oral papilloma ve skuamöz hücreli karsinoma tanısı konuldu. İmmunohistokimyasal incelemede neoplastik hücrelerde p16 proteini için sitoplazmik ve nükleer pozitif reaksiyonlar gözlendi. Daha sonra vincristine, antibiyotik, radyoterapi ve yüksek C vitamini dozlarının kombine tedavisi uygulandı. Uzun süreli bir tedaviden sonra, hastada izlenen lezyonlar tamamen iyileşti. Bu olguda papillomanın oral skuamöz hücreli karsinom gelişimindeki potansiyel rolü ile birlikte klinik, histopatolojik, immünohistokimyasal bulgular ve tedavi prosedürünün sunulması amaçlanmıştır.

Anahtar sözcükler: İmmunohistokimya, köpek, oral papilloma, skuamöz 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 seropositive 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- $p16^{INK4a}$ (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

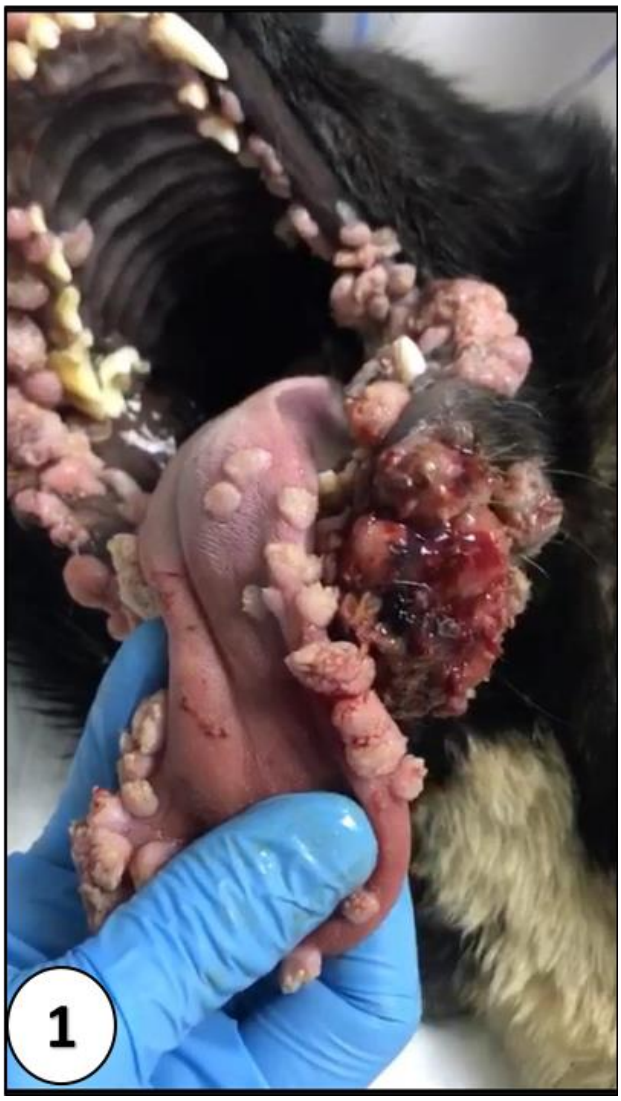

Figure 1. Pinkish, pedunculated, cauliflower-like exophytic masses on the lateral surface of the tongue and in the entire oral cavity.

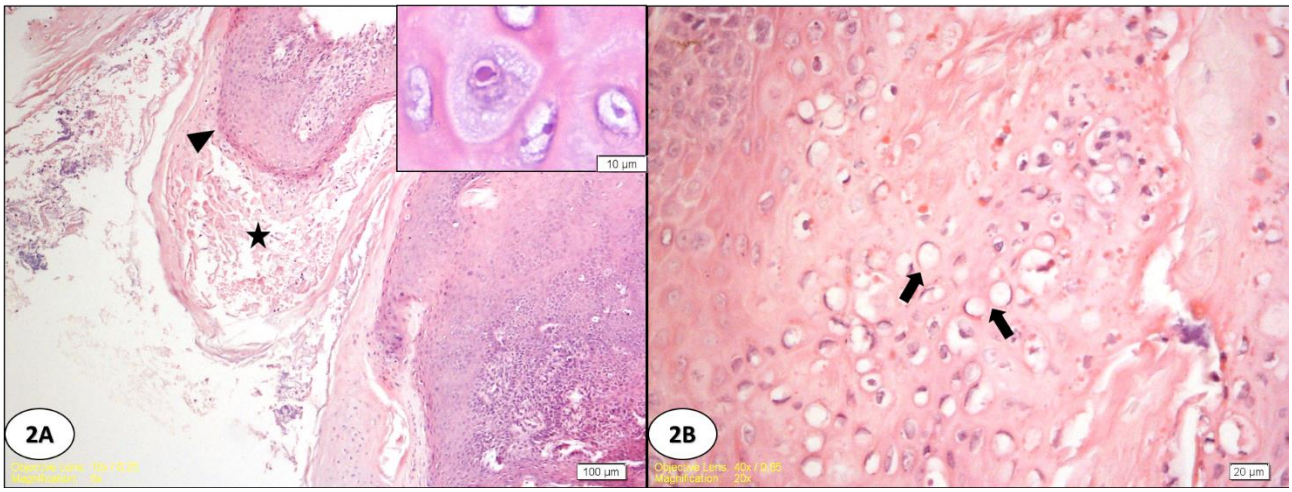

Figure 2 A. The appearance of papilloma with severe papillomatous hyperplasia (arrowhead) within the mucosal epithelium and hyperkeratosis (star). H&E. Bar = 100 µm. **B**. Numerous koilocytes with a swollen nucleus surrounded by a clear halo (arrows). H&E. Bar = 20 µm. Intra-nuclear eosinophilic inclusion bodies in the epithelial cell (2A, inset). H&E. Bar = 10 µm.

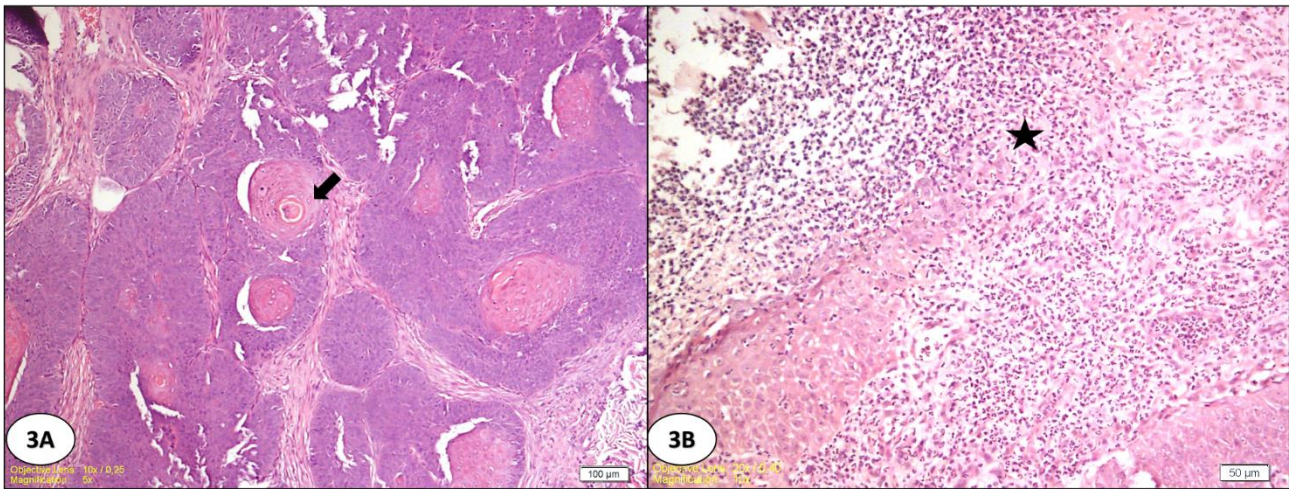

Figure 3 A. High-grade epithelial hyperplasia from the mucosa to the submucosa in the canine oral cavity. Neoplastic differentiation characterized by different degrees of keratinization (horn pearls /arrow) in squamous epithelial cells clustered as nests. H&E. Bar = 100 µm. **B.** Superficial ulceration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

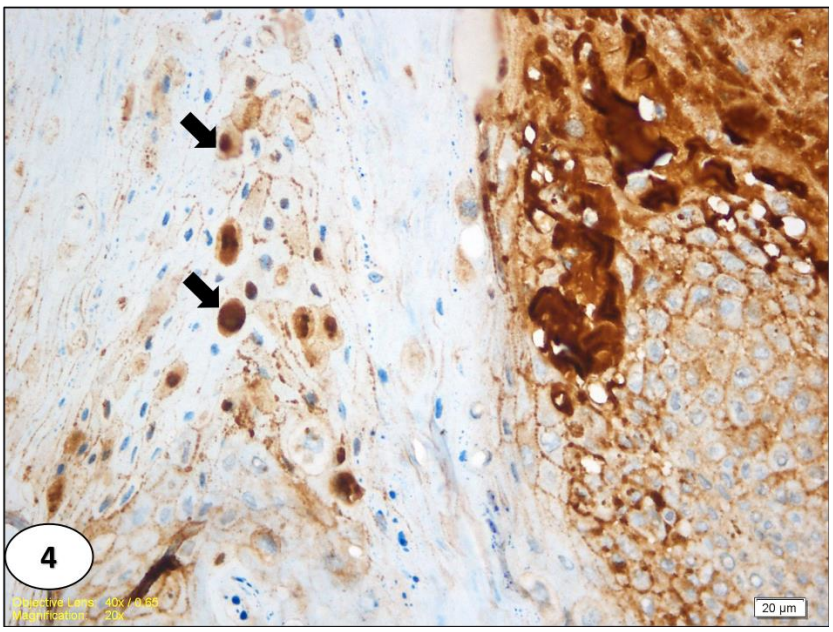

Figure 4. Positive immune reaction against P16 antigen in neoplastic canine oral SCC (arrows). IHC with Anti-p16^{INKA4a}(E6H4). Bar = 20 µm.

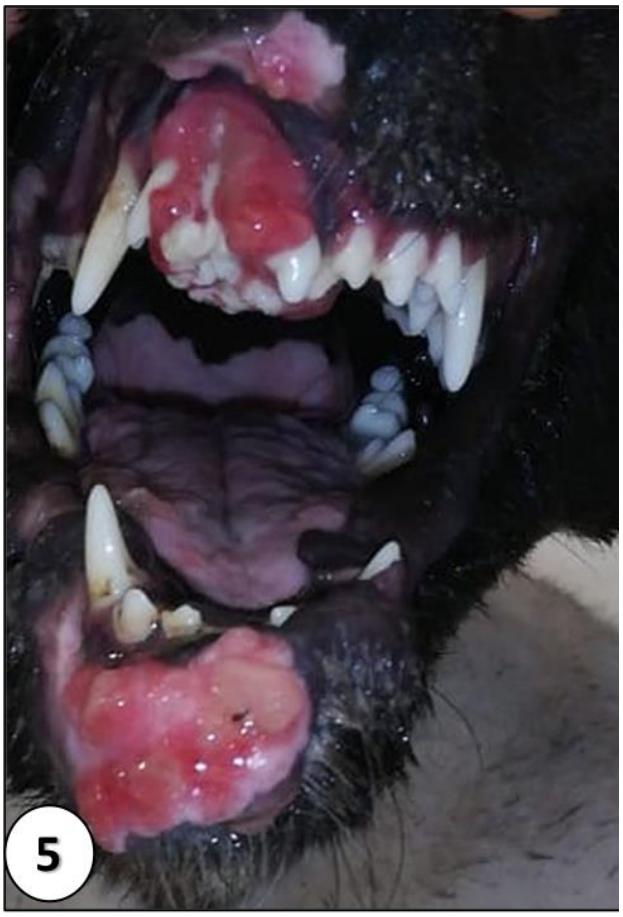

Figure 5. Decreased PV lesions after the treatment with vincristine and prominent ulcerative SCC in the oral cavity.

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.

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Ethical Statement

This study does not present any ethical concerns.

Conflict of Interest

The authors declared that there is no conflict of interest.

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