A case of papillary and infiltrative urothelial carcinoma of the urinary bladder in a terrier dog

ABSTRACT

Urothelial carcinomas are malignant tumours originating from the epithelial layer of the urinary bladder. In this instance, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a 2-year-old terrier dog was defined clinically, histopathologically and immunohistochemically. The material of the presented case consisted of urinary bladder tissue samples that were surgically extirpated from a two-year-old terrier-breed female dog that applied to the Department of Surgery of the Faculty of Veterinary Medicine, Selcuk University with the complaint of hematuria. The tumour brought to the pathology laboratory was 11x10x12 cm in size and had finger-shaped extensions. Its outer surface was rough and hemorrhagic. Tissues were fixed in 10% buffered formalin and paraffin blocks were obtained by going through the necessary routine follow-up procedures. Afterwards, sections were taken and subjected to Hematoxylin-Eosin, Masson's Trichrome and immunohistochemical staining. As a result of the pathological and immunohistochemical examinations of the tumoral tissue samples taken from the urinary bladder, the diagnosis of papillary and infiltrative urothelial carcinoma was reached, and the case was discussed with the information provided by the literature. In addition, immunohistochemically, intense Proliferating cell nuclear antigen (PCNA) and Vascular endothelial growth factor (VEGF) staining has been associated with malignancy.

Keywords: Urinary bladder, urothelial carcinoma, dog, histopathology

INTRODUCTION

The prevalence of urinary bladder tumours is quite low in dogs. They constitute only 0.5-1% of the tumours seen in dogs. It has been reported that almost all urinary bladder tumours have malignant features, and approximately 97% are of epithelial origin (Güzel, 2006; Maxie, 2015; Meuten, 2017). Urothelial carcinomas are malignant tumours originating from the epithelial layer of the urinary bladder. In the urinary system, the urinary bladder is the area where malignancy most commonly occurs (Meuten, 2017). This is probably due to the prolonged contact of the urinary bladder with carcinogens excreted through the kidneys and found in the urine (Maxie, 2015; Meuten, 2017; Yönez, 2016). The most common urinary bladder tumour in dogs is urothelial carcinoma. It is mostly seen in dogs of 11 years and older. Urothelial carcinomas are especially seen in older female dogs weighing more than 10 kg and exposed to benzene-containing insecticides (Fulkerson and Knapp, 2015; Knapp et al., 2014; Meuten, 2017; Moulton, 1990; Norris et al., 1992).

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Case Report

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Blood vessels are required for the invasion and metastasis of a solid tumour. There are many growth factors in tumour angiogenesis, especially vascular endothelial growth factors (VEGFs) (Haigh et al., 2000). Studies on tumour prognosis focus on VEGF, and it is defined as a critical regulator of angiogenesis (Sia et al., 2014; Simons et al., 2016).

Proliferative cell nuclear antigen (PCNA) is a nuclear protein that participates in DNA synthesis, is synthesised in the normal cell cycle and plays a role in the regulation of the cycle. PCNA, first synthesised in the G1 phase, reaches its peak level in the S phase. Afterwards, it decreases significantly in the M and G2 phases (Maga and Hubscher, 2003; Pradhan et al., 2019). PCNA is frequently used as a marker of cell proliferation in healthy and tumour tissues (Tehseen et al., 2019). In addition, it is stated that neoplastic cells are closely related to their biological activities and play an effective role in carcinogenesis, and there is a positive correlation between PCNA expression and malignancy (Ahmed and Sozmen, 2020; Ozdemir et al.2022; Ye et al., 2020).

In this instance, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a 2-year-old terrier dog is defined clinically, pathologically, and immunohistochemically. It was deemed appropriate to publish the case because of its uncommonness.

CASE REPORT

The material of the presented case consisted of urinary bladder tissue samples that were surgically extirpated from a two-year-old terrier-breed female dog that applied to the Department of Surgery of the Faculty of Veterinary Medicine, Selcuk University with the complaint of hematuria. From the anamnesis, it was learned that the patient, even though frequently adopting the urination position, could only urinate with a few drops of bloody urine, that there was no change in appetite and body condition, and that these symptoms had been observed for approximately a month. In the macroscopic examination of the urine taken with the catheter, it was determined that it contained dirty yellow turbid colour and sediment. In the examination of urine centrifuged at 1000 g for 5 minutes, hematuria was detected. A drop of the centrifuged urine sample was taken from the sediment on a slide, and a microscopic examination was performed. The microscopic examination revealed that erythrocytes, leukocytes, urine crystals and epithelial cells were found in masses in each microscope field. During the operation, it was noted that tumour structures were also found in the serosa of the urinary bladder and the adipose tissue surrounding it. In addition, it was determined that the tumour formed plaques spreading to all layers of the urinary bladder wall and made papillary extensions connected to the mucosa, reaching into the urinary bladder.

The tumour brought to the pathology laboratory was 11x10x12 cm in diameter and had finger-shaped extensions. Its outer surface was rough and hemorrhagic. Although the finger-shaped masses presented a red appearance, it was determined that some areas had grey-white foci too. The masses with a hard consistency had a whitish pink colour on the cross-sectional area and included hemorrhagic areas (Figure 1). Tissues were fixed in 10% buffered formalin. After routine follow-up procedures, 4-5 µm thick sections were taken from the prepared paraffin blocks. Sections were stained with Hematoxylin and Eosin (H&E), Masson's Trichrome methods and examined under a light microscope. Immunohistochemistry staining was performed according to Tuzcu et al. (2022). Paraffin extraction and rehydration processes were performed on the sections. The antigen retrieval process was performed by boiling in citrate buffer solution (pH: 6) for 2x5 minutes at 500 watts. Immunohistochemistry staining was performed with the UltraVision Detection

System Anti-Polyvalent, HRP (Ready-to-Use, TP-060-HL, Lab Vision, USA) ihc kit following the recommendations of the manufacturer. Monoclonal Anti-PCNA (1:200, Dako, clone PC10, M0879, 1-hour incubation) and monoclonal Anti-VEGF (1:200, Santa Cruz Biotechnology, sc-7269, 1-hour incubation) were used as primary antibodies. 3.3 diaminobenzidine (DAB) was used as chromogen and counterstained with Mayers Hematoxylin. It was then passed through a series of alcohol and xylene, covered with a coverslip and examined under a light microscope (Olympus BX51, Tokyo, Japan).

In the microscopic examination, it was observed that tumoral growths made papillary extensions from the mucosa to the lumen, as well as growths in the propria and significant connective tissue formation (desmoplasia) around them. It was determined that different microscopic areas of the tumour mass differed in terms of malignancy criteria. It was noted that in some sections examined; in the neoplastic cells, had nuclei of varying sizes and randomly ordered in a abundant eosinophilic cytoplasm, polarity was completely lost, marked atypia and pleomorphism, along with increased mitotic figures, were observed (Figure 2. A-C). Papillary extensions with urothelial proliferation were noted in some regions. In various zones, it was observed that tumour cells were surrounded by connective tissue. In addition, signet ring cells and Melamed wolinska bodies were detected sporadically (Figure 2. B-C). Furthermore, inflammatory cell infiltrations were also detected in the sections along with foci of necrosis (Figure 2. B-C). It was determined that the blood vessels were filled with erythrocytes (congestion, Figure 1. A, Figure 2. A-D), and sporadically there were bleedings. The Masson's Trichrome staining showed that a large connective tissue surrounded the tumour masses located on the urinary bladder wall (Figure 2. D). In the staining with VEGF, significant immunopositivity was observed in both neoplastic cells (cytoplasmic) and endothelial cells in the regions (Figure 3. C-D). Intense nuclear immunopositivity was determined in atypical tumour cells in staining with anti-PCNA (Figure 3. A-B).

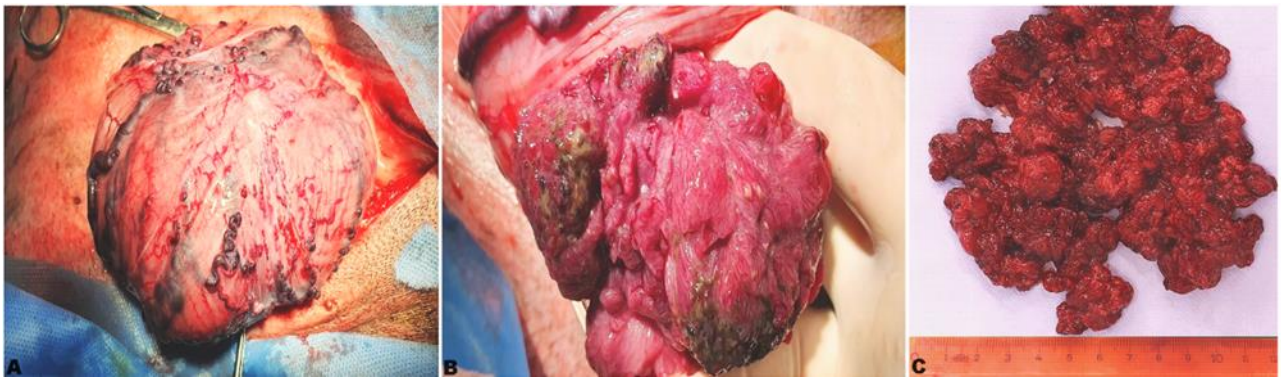

Figure 1. A. Tumor foci in the serosa of the urinary bladder, **B.** Tumor foci formed in the mucosa of the urinary bladder, **C.** Macroscopic view of the surgically removed tumor tissue

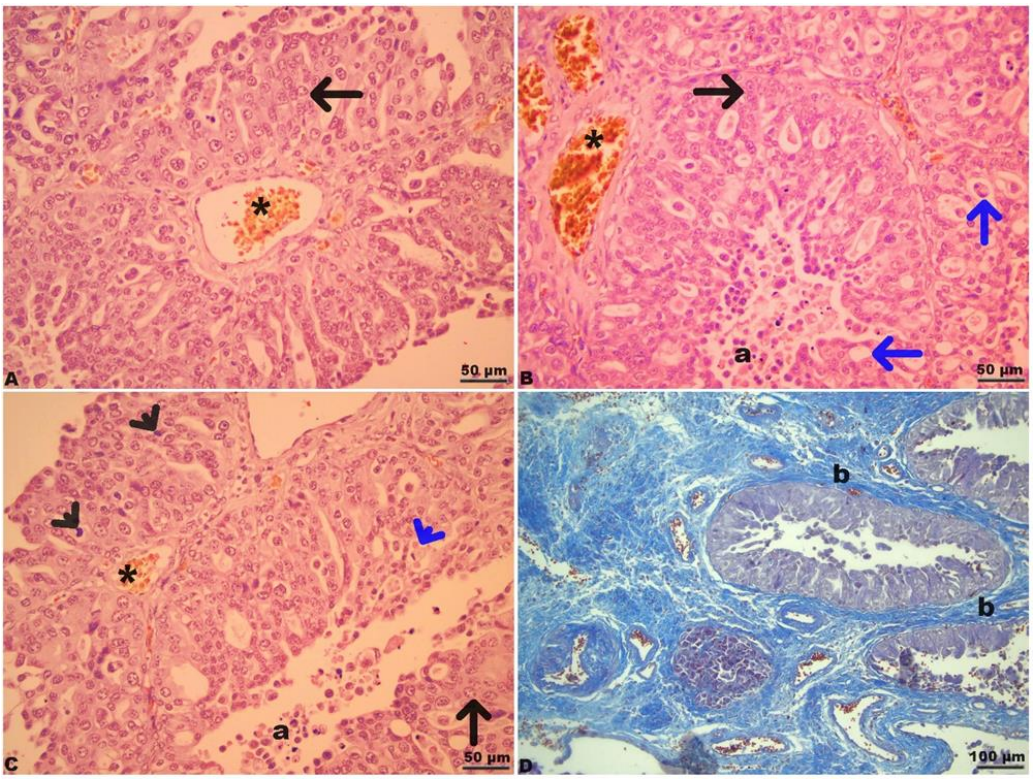

Figure 2. A-B-C. Histopathological examination of the tumoral mass, H&E, hyperemia (star), atypical cell features (black arrow), epithelial cells spilled into its lumen, and inflammatory cell infiltrates (a), mitotic figures (black arrowhead), signet ring cells (blue arrow), Melamed-wolinska bodies (blue arrowhead). **D.** View of the connective tissue surrounding the tumor mass (b), Masson's Trichrome

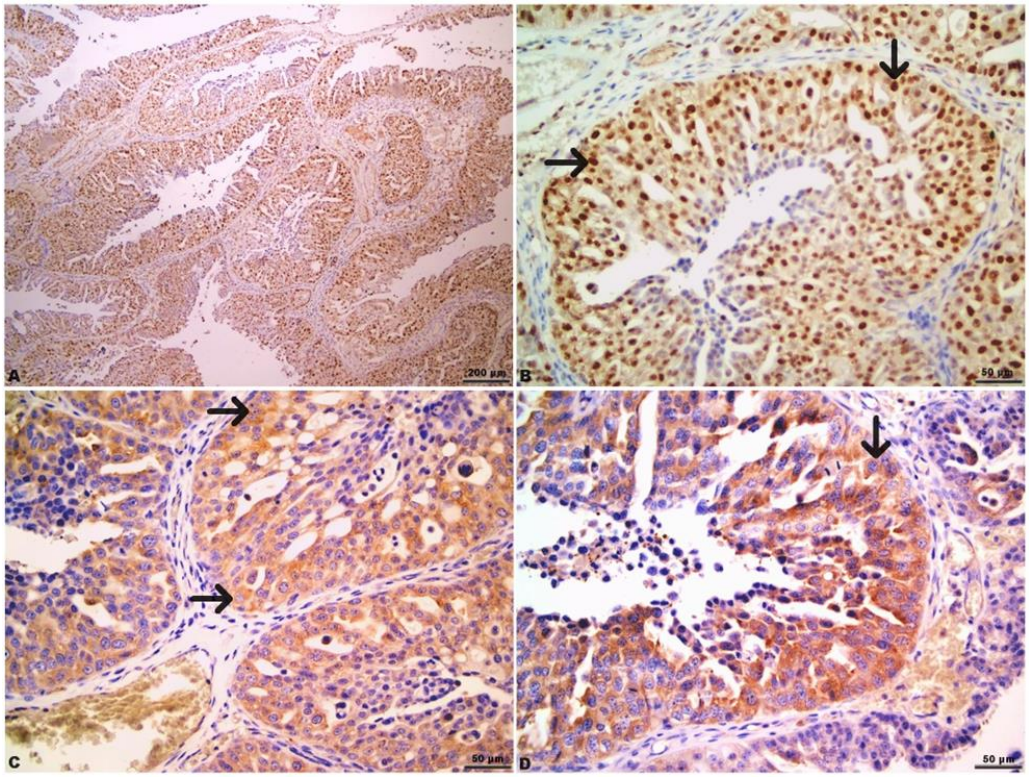

Figure 3. Anti-PCNA immunohistochemical staining (DAB), dense nuclear immunopositive cells (**A-B**) in atypical and other cells in the tumoral area (arrow). Anti-VEGF immunohistochemical staining (DAB), intense cytoplasmic staining (**C-D**) of cells in the tumoral area (arrows).

DISCUSSION

In the presented report, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a terrier dog is described clinically and pathologically. Since this was the first case encountered in our laboratory and the dog was young, it was deemed appropriate to publish it.

Urothelial carcinomas are divided into four groups according to their macroscopic and microscopic appearances as non-papillary and non-infiltrating carcinoma, papillary carcinoma, papillary and infiltrative carcinoma, and infiltrative carcinoma (Maxie, 2015; Meuten, 2017). When the macroscopic and microscopic findings of the presented case were evaluated, they were consistent with the findings of papillary and infiltrative urothelial carcinoma.

Studies indicate that the incidence of tumours in dogs increases with age (Kent et al., 2018; Kok et al., 2019; Yönez, 2016). Although it has been reported that urinary bladder tumours are mostly seen between the ages of 4 and 16, it is informed that these tumours develop on average around 9-10 years of age (Knapp et al., 2014; Meuten, 2017; Moulton, 1990; Strafuss and Dean, 1975). In this case, it was noteworthy that the dog was 2 years old. The appearance of this tumor at a young age can be interpreted as breed susceptibility of terrier dogs to this tumor (Meuten 2017), although it is not certain.

Urothelial carcinomas are single or multiple. The latter presents a differing appearance among them, and they have mostly a papillary structure. Polyp-like and broad-based tumours may also be encountered. They often vary in size, and some fill the bladder completely (Maxie, 2015; Meuten, 2017; Norris et al., 1992). In the presented case, plaques infiltrated into the urinary bladder wall were detected next to the papillary structures. In addition papillary structures were observed filling the urinary bladder, similar to the literature.

It is stated that for the diagnosis of urothelial carcinoma, anaplasia, invasion of the urinary bladder wall, and metastasis in tumour cells should be evaluated (Meuten, 2017). In dogs, urothelial carcinomas metastasis to the regional lymph nodes and lung are encountered in the late stage, along with peritoneal implants or retrograde lymphatic spread to soft tissues and hind leg bones also (Fulkerson and Knapp, 2015; Meuten, 2017; Moulton, 1990). In the presented case, anaplasia and invasion into the sac wall were evident in tumour cells, similar to the literature. Tumour structures located in the serosa of the urinary bladder were determined, whereas metastases in neighbouring organs could not be determined.

In the presented case, it was determined that different microscopic areas of the tumour mass were different regarding malignancy criteria. In some sections examined, it was noted that the nuclei were randomly ordered and of different sizes, while a significant atypia and pleomorphism were found in the tumor-forming cells. While papillary extensions with urothelial proliferations were observed in some areas, it was observed that tumor cells were surrounded by connective tissue in some areas. The microscopic findings determined in this case were consistent with the literature (Mantovani et al., 2006; Maxie, 2015; Meuten, 2017). Necrosis, which is known as a characteristic of malignant tumours (Erer and Kıran, 2009), was observed in the form of foci ranging from small areas of necrosis formed by two or three cells to large areas of necrosis that can be noticed macroscopically.

Restucci et al. (2003), reported that they found VEGF expression to be higher in canine seminomas compared to the control group and reported that it was a useful criterion regarding malignancy and growth potential in seminomas. Campos et al. (2012) reported that VEGF levels increase significantly in hemangiosarcomas and are associated with malignant vascular

proliferation. Martano et al. (2016) reported that VEGF expressions were significantly increased in neoplastic tissues compared to normal tissues in a study on oral squamous cell carcinomas of dogs. In the presented case, the very prominent staining of VEGF in neoplastic tissues and vascular endothelium was consistent with the literature showing a correlation between malignancy and VEGF.

Krishna et al. (2022), in a study on mammary tumours in dogs, reported that PCNA scores increased in malignant tumours and were associated with malignancy. Aydogan et al. (2018) stated that PCNA expression has prognostic value in canine mammary tumours. In the study conducted by Ahmed and Sozmen (2020) on feline and canine fibrosarcomas, it was reported that PCNA expressions increased as the tumour malignancy grade increased. In the present case, intense nuclear immunopositivity was detected in atypical tumour cells in PCNA staining, which is consistent with the literature showing a correlation between malignancy and PCNA.

CONCLUSION

In conclusion, in this case, the diagnosis of papillary and infiltrative urothelial carcinoma was reached as a result of the clinical, pathological and immunohistochemical examinations of tumour samples taken from the urinary bladder of a two-year-old terrier dog, and the case was discussed with the literature. In addition, intense PCNA and VEGF staining immunohistochemically has been associated with malignancy.

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Ethical approval:

Ethical committee is not required as it consists of inanimate material (SÜVDAMEK, directive number 10.4)

Conflict of interest:

The authors declared that there is no conflict of interest.

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