Case Report

Partial Cystectomy and Ureteroneocystostomy in a Dog with Urinary Bladder Squamous Cell Carcinoma

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Abstract

Squamous cell carcinoma (SCC) is a rare type of urinary bladder carcinoma in dogs. A 10-year-old spayed bitch was presented with pollakiuria. Ultrasonography revealed right obstructive hydronephrosis and proximal hydroureter. Urinary bladder wall thickening and an irregular mucosal surface were presented. Partial cystectomy and ureteroneocystostomy of the right ureter were performed for urinary bladder mass removal in the area close to the right ureteral opening. The histopathological characteristics of urinary bladder SCC were confirmed by negative immunohistochemical staining of uroplakin III, cytokeratin 7, and cytokeratin 20. Two months after surgery, multiple nodules in all hepatic lobules were presented from ultrasonography. The distant metastasis was suspected. This report demonstrates the diagnostic approach and management of urinary bladder SCC, a rare type of canine urinary bladder cancer.

Keywords: Bladder cancer, Dog, Partial cystectomy, Squamous cell carcinoma

การตัดกระเพาะปัสสาวะออกบางส่วนร่วมกับเปลี่ยนย้ายรูเปิดท่อไตบริเวณกระเพาะปัสสาวะในสุนัขที่เป็นมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์

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บทคัดย่อ

สุนัขเพศเมียที่ทำหมันแล้ว อายุ 10 ปี เข้ารับการรักษาที่โรงพยาบาลสัตว์ด้วยอาการปัสสาวะบ่อย จากการวินิจฉัยด้วยการทำอัลตร้าซาวด์พบว่า ไตข้างขวาและท่อไตส่วนต้นด้านขวาบวมร่วมกับผนังกระเพาะปัสสาวะมีการหนาตัวและลักษณะเยื่อบุด้านในไม่เรียบ จากการผ่าตัดพบว่ามีก้อนเนื้องอกที่กระเพาะปัสสาวะจึงได้มีการตัดก้อนเนื้องอกและกระเพาะปัสสาวะที่ใกล้กับตำแหน่งรูเปิดท่อไตด้านขวาออกบางส่วนร่วมกับเปลี่ยนย้ายรูเปิดท่อไตด้านขวา ผลการตรวจทางจุลพยาธิวิทยาพบว่าเป็นมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์ และมีการยืนยันผลการวินิจฉัยนี้ด้วยการทดสอบทางอิมมูโนฮิสโตเคมีซึ่งให้ผลการย้อมแอนติเจนยูโรไพคิน 3 (uroplakin III) ไซโตเคราติน7 (cytokeratin7) และไซโตเคราติน20 (cytokeratin20) เป็นลบ อย่างไรก็ตามภายหลังการผ่าตัดเป็นเวลา 2 เดือนได้มีการอัลตร้าซาวด์พบก้อนที่ตับ จึงสันนิษฐานว่าเป็นการแพร่กระจายไปยังอวัยวะอื่น ข้อมูลรายงานสัตว์ป่วยนี้จะนำเสนอการผ่าตัดกรณีของมะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์ซึ่งเป็นมะเร็งชนิดที่พบได้ยากในสุนัข

คำสำคัญ : มะเร็งกระเพาะปัสสาวะ สุนัข การผ่าตัดกระเพาะปัสสาวะออกบางส่วน มะเร็งกระเพาะปัสสาวะชนิดสความัสเซลล์

Introduction

Canine urinary bladder neoplasia, which accounts for 2% of all cancers, can result in partial or complete obstruction of the urinary tract. The most frequent type of bladder cancer is transitional cell carcinoma (TCC) (Fulkerson and Knapp 2019), while squamous cell carcinoma (SCC) is less common in dogs with a 1.5 - 2% incidence of bladder cancers (Ramos-Vara et al., 2003; de Brot et al., 2018). To present, in Thailand, only urinary bladder TCC (Rungsipipat et al., 2003; Suwankanit and Manee-in 2018) and urinary bladder leiomyoma have been reported (Ploypetch et al., 2021). Secondary cancer on the urinary bladder has rarely been observed. Studies in humans have found that bladder SCC usually occurs in the trigone area as well as the urethra or ureters. Moreover, common sites of metastasis of SCC of the urinary bladder are the lung, bone, and liver (Manley et al., 2017). Surgery, radiation, chemotherapy, medicinal therapy or a combination of these are used to treat bladder cancer in humans and dogs (Zahoor et al., 2018; Burgess and DeRegis 2019; Fulkerson and Knapp 2019). Radical cystectomy is a conventional treatment for human bladder SCC, as it provides better disease control and recurrence-free survival time than other treatments, when combined with radiotherapy (Richie et al., 1976; Martin et al., 2016).

This case study illustrates the surgical management of urinary obstruction due to urinary cancers, and the SCC process to confirm the diagnosis.

Case Description

A 10-year-old spayed bitch American Cocker Spaniel with myxomatous mitral valve disease stage B2 was referred to Prasu-Arthorn Animal Hospital, Mahidol University for evaluation of anorexia and pollakiuria. The vital signs were normal, except for a grade 4/6 murmur heart sound. Blood profiles were within the reference interval, as shown in Table 1. Serum biochemical analysis showed hyperproteinemia (8 g/dl, reference 6 - 7.5 g/dl), slightly increased alanine aminotransferase (265 IU/l, reference 10 - 100 IU/l), and high blood urea nitrogen (55 mg/dl, reference 7-27 mg/dl). The cystocentesis urinalysis results revealed a slightly transparent, yellow, inappropriate concentration of urine (urine specific gravity 1.010) and pH 7. Proteinuria was also present, which was detected by urine strip testing. Due to some bacteriuria, a urine sample was taken for bacterial identification and antibiotic susceptibility testing. *Proteus mirabilis* sensitive to cephalosporins was interpreted as the bacterial culture and antimicrobial sensitivity result. Thoracic and abdominal radiography did not present any visible abnormalities. Abdominal ultrasonography showed right hydronephrosis (Figure 1A). The right renal pelvis dimension is 1.6 x 2.1 cm with a transverse view (Figure 1B). The right proximal ureter was obstructed and dilated (Figure 1C) with a hyperechoic structure. The urinary bladder had wall thickening, with an irregular mucosal surface pronounced at the right apex part (Figure 1D). Therefore, the patient was diagnosed with right outflow obstructive hydronephrosis due to early ureteral obstruction. Surgical treatment to correct the right ureteral obstruction was planned.

Table 1. Hematology report (absolute) from the first visit and 7 days after surgery.

Parameters*	First visit	Seven days after surgery	Normal value
WBC (µl)	11,160	9,500	6,000 - 17,000
Monocyte	669.6	475	150 - 1,350
Neutrophil	8,370	6,745	300 - 11,500
Band	0	0	0 - 300
Lymphocyte	2,120.4	2,185	1,000 - 4,800
Eosinophil	0	95	100 - 1,250
Basophil	0	0	<100
RBC (10^6 cell/µl)	6.65	7.05	5 - 9
Hb (g/dl)	16	17.0	10 - 18
Hct (%)	47.9	50.7	35 - 55
MCV (fL)	72	71.9	60 - 77
MCH (pg)	24.1	24.1	20 - 25
MCHC (g/dl)	33.4	33.5	32 - 36
PLT (10^3/µl)	197	213	200 - 500
Platelet smear	Adequate	Adequate	
Plasma Protein	8	8	6.0 - 7.5
ALT (U/L)	265	255	10 - 100
ALP (U/L)	107	118	23 - 212
BUN (mg/dL)	55	50	7 - 27
Creatinine (mg/dL)	1.36	1.44	0.5 - 1.8

*WBC: White blood cells; RBC: Red blood cells; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelets; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; BUN: Blood urea nitrogen.

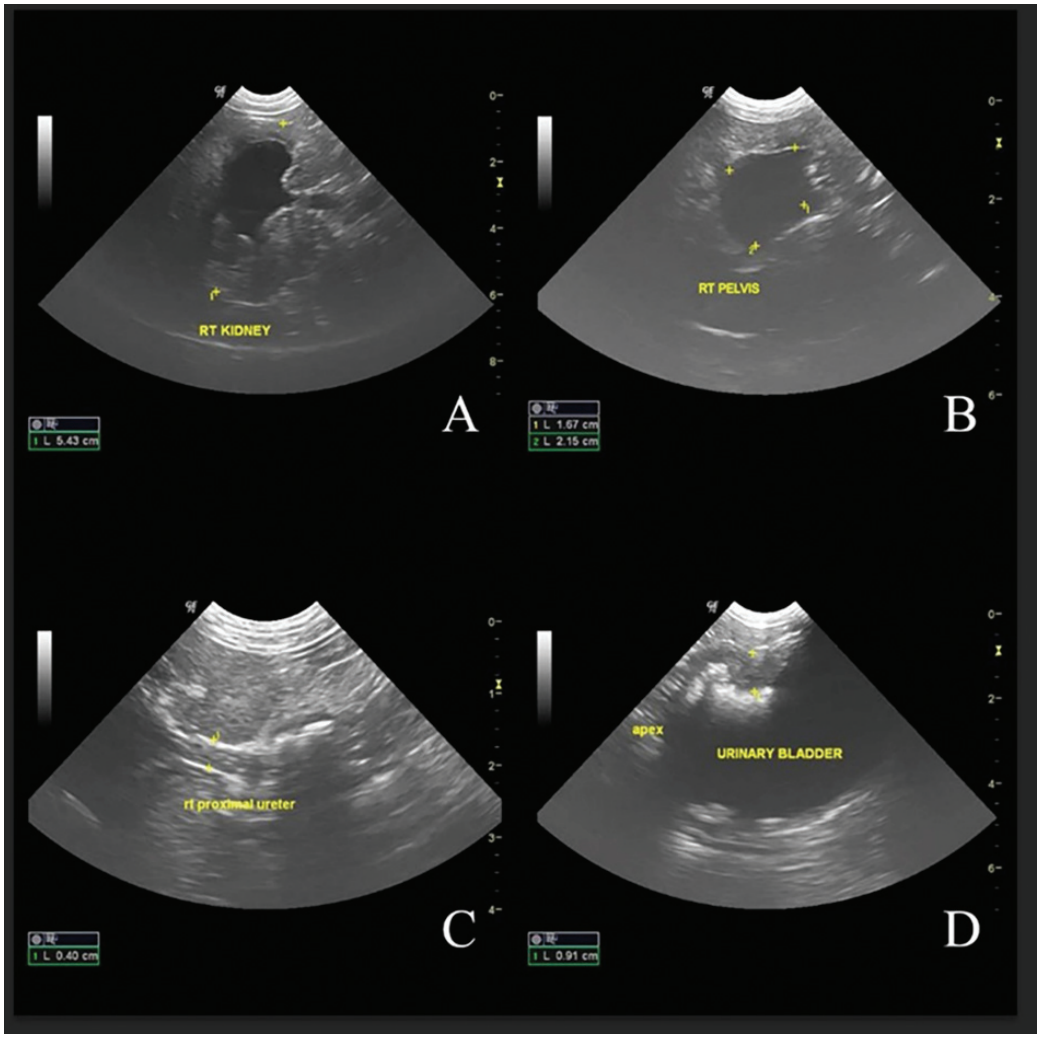


Figure 1. Abdominosonographic findings. (A) Right hydronephrosis. (B) The dilatation of the right renal pelvis. (C) Dilated right proximal ureter. (D) The urinary bladder has wall thickening with an irregular mucosal surface pronounced at the apex part.

The patient underwent a partial cystectomy at the right ureter orifice, as well as a right ureteroneocystostomy. Anaesthetic drugs were selected to minimize the cardiac and renal side effects of general anaesthesia. In this case, fentanyl (3 μg/kg fentanyl-Hameln; Siam Bioscience Co. Ltd., Thailand) and midazolam (0.3 mg/kg midazolam; F. Hoffmann-La Roche Ltd., Switzerland) were intravenously administered as a premedication, then inducted with alfaxalone (2 mg/kg Alfaxan®; Jurox Pty. Ltd., Australia). An injectable drug with morphine (0.3 mg/kg morphine sulphate injection; M&H Manufacturing Co. Ltd., Thailand) was injected intramuscularly for analgesia and amoxicillin/clavulanic acid (20 mg/kg Cavumox®; Siam Bheasach Co. Ltd., Thailand) was intravenously administered for anti-microbial prophylaxis. The patient was intubated and maintained at an anaesthetic level using isoflurane (AERRANE; Baxter Healthcare of Puerto Rico, United States) inhalation with oxygen. The patient was positioned in dorsal recumbency. A ventral midline celiotomy was performed through the caudal aspect of the abdominal wall with a ventral midline cystotomy. Intraoperation, a mass that involved the right

ureteral opening and bladder apex was presented, causing right ureteral dilatation (Figure 2A). Partial cystectomy was then performed, respecting a margin of 20 mm on the sides. Resection comprised about 40 to 50% of all bladder tissue (Figure 2B). The right ureter was removed approximately 5 mm. A ureteroneocystostomy was done with an interrupted pattern using absorbable 4-0 polydioxanone suture material (PDS™ II 4-0; Johnson & Johnson Medical N.V., Belgium) (Figure 2C) to reimplant the right ureter into the bladder. The bladder wall was closed with a simple continuous pattern using absorbable 3-0 poliglecaprone 25 suture material (Monocryl™ 4-0; Johnson & Johnson Medical N.V., Belgium). Finally, the abdomen wall was closed utilizing the routine technique. A postoperative urinary catheter was placed to monitor urine output for 7 days. The bladder mass (Figure 2D), measuring 18 mm (length) x 15 mm (width) x 15 mm (height), was examined histopathologically and stained with haematoxylin & eosin (H&E) and immunohistochemistry (IHC) performed.

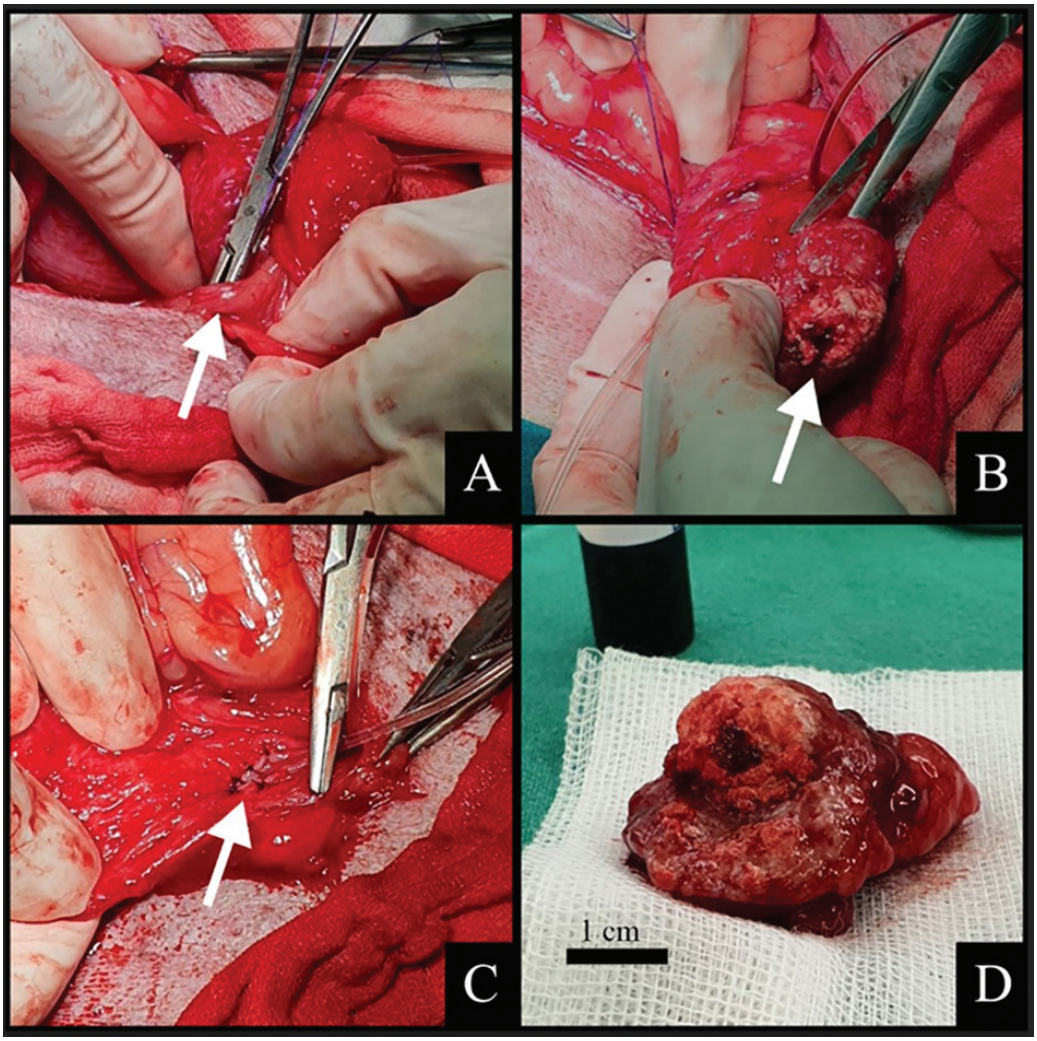


Figure 2. Ureteroneocystotomy and partial cystectomy procedure. (A) A laparotomy found right ureteral dilatation (arrow). (B) Partial cystectomy for removal of the entire mass (arrow). (C) Ureteroneocystotomy for reimplantation of the ureter on the surface of the bladder (arrow). (D) Excised mass with rough shape and irregular surface.

Microscopically, the mass revealed multifocally non-encapsulated, well-differentiated, strongly proliferative, pleomorphic squamous cells in the submucosal layer and invading through the transitional epithelial layer. Pleomorphic cells were arranged in cords to islets of proliferative squamous cells. Squamous cells were hyperplastic with anisocytosis, round, of cuboidal to polyhedral shape with abundant amphophilic cytoplasm with occasional intercellular bridges. Nuclei had anisokaryosis, were vesiculated oval to round with prominent nucleoli. Mitotic figures were occasionally noticed. Eosinophilic keratin precipitation was occasionally detected in cords. Submucosal layers were occupied by hyperplastic fibrous tissues with focally extensive submucosal layers. There was no evidence of metastasis. Keratin pearl was well differentiated for SCC diagnosis (Figure 3A). The streptavidin-biotin peroxidase technique with commercial kits was used for immunohistochemistry. The primary antibodies including Uroplakin III (UR III) (1: 450 Clone AU-1; Cell Marque, USA), cytokeratin 7 (CK7) (1: 1,000 Clone OV-TL 12/30; Cell Marque, USA(, and cytokeratin 20 (CK 20) (1: 3,000 Clone Ks20.8; Cell Marque, USA) were used to determine SCC of the urinary bladder. Only the apical border of the cytoplasmic membrane of transitional cells showed strongly intense positivity for three markers. The intermediate and superficial cells were also cytoplasmically stained. The lower cells and basal area were negative. The dysplasia of proliferative squamous cells had a lack of immunoreactivity. All negative immunohistochemistry (Figures 3B, 3C, and 3D) results confirmed the presence of SCC.

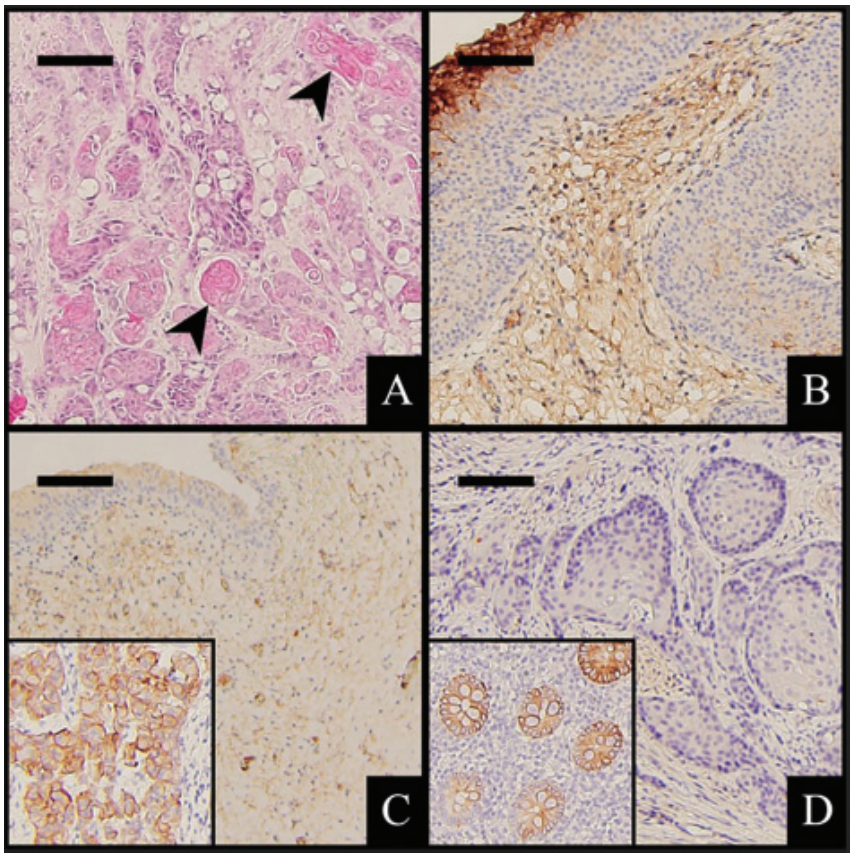


Figure 3. Immunohistopathologic findings. (A) The rounded-looped shapes are diagnosed as keratin pearl (arrowheads) from H&E staining. Immunohistochemical staining of UR III (B), CK 7 (C), and CK 20 (D). The positive controls of CK 7 and CK 20 are shown as an inset in the lower left corner of (C) and (D), respectively. Bar = 200 μm.

Afterwards, the patient was admitted to the hospital for 7 days for post-operative care. Vital signs, especially blood pressure and urine output, were closely measured. Moreover, the post-operative practices including fluid therapy, antimicrobials, analgesics, wound dressing and recheck blood profiles were performed in the critical care unit. For pain management, an injectable drug with morphine (0.3 mg/kg morphine sulphate injection; M&H Manufacturing Co. Ltd., Thailand) was injected subcutaneously every 8 hours, depending on the pain score. No clinical signs of the urinary system were observed 7 days after surgery. Blood profiles were rechecked before discharge from the hospital. Hematologic values were showed in Table 1.

Ultrasonography was used to check for renal hydronephrosis and ureteral dilation, as well as the recurrence of cancer one month after surgery. The dilatation of the right renal pelvis and right proximal ureter was reduced when compared with before surgery. The urinary bladder's wall thickness was normal. The right ureteral opening of the bladder had a mucosal surface that was slightly irregular and assumed fibrosis following ureteroneocystostomy. Although the dog showed no clinical signs, ultrasonography in two months after surgery revealed multiple nodules in all hepatic lobules and left medial iliac lymphadenopathy. Metastasis was suspected. Metronomic chemotherapy with a combination of chlorambucil (4 mg/m2 LeukeranTM; Aspen Pharmacare Australia Pty Ltd., Australia) and firocoxib (2 mg/kg Previcox®; Boehringer Ingelheim, Germany) was used to improve the quality of life. No side effects from this low-dose chemotherapy were observed in this case. Moreover, the owner described that no clinical signs of haematuria or trouble urinating had appeared at least 10 months after metronomic administration.

Discussion

According to the universal consensus in humans, radical cystectomy is required for the surgical treatment of urinary bladder SCC. Recurrence-free survival rates for the entire patient population after radical cystectomy at 5 and 10 years were 68 and 66%, respectively (Stein et al., 2001). On the contrary, previous studies in dogs that received this technique had many complications, including pyelonephritis, peritonitis, oliguria, azotaemia and ureteral obstruction (Ricardo Huppes et al., 2017). A partial cystectomy with extensive resection, which was analogous to traditional surgery for removing urinary bladder carcinoma, was undertaken alternatively. Except for tumour metastasis, no complications were discovered during or after surgery. However, the presence of residual tumour at the site of resection through neoplastic transformation of the remaining bladder mucosa and implantation of tumour cells into the surgical wound, resulting in local tumour recurrence, might be a common consequence after bladder SCC resection (Zahoor et al., 2018). The study in humans undergoing partial cystectomy for bladder cancer reported that bleeding, infection, reduced bladder capacity, and urinary extravasation are all common consequences after surgery (Kates et al., 2014).

Once distinguishing characteristics such as keratin pearls and intercellular bridges have been recognized, SCC can be definitively confirmed histologically. Moreover, these features can only be seen when the SCC is still a well-differentiated neoplasm (Lopez-Beltran et al., 2007). Despite the presence of keratin pearls and intercellular bridges, this case was also diagnosed using the IHC technique. For the detection of canine urothelial cancers, UP III is more specific than CK 7 and CK 20. Between SCC and TCC, the immunohistochemistry staining

patterns of CK 20, CK 7 and UP III are significantly different. All these markers are negative in bladder SCC as in this case (Ramos-Vara et al., 2003). Therefore, this case was definitively diagnosed as canine urinary bladder SCC, confirmed by histopathology and the IHC technique.

Regarding the treatment of bladder SCC, combining preoperative radiation with radical cystectomy resulted in a significantly greater 5-year survival rate for humans than any other treatment (Martin et al., 2016; Manley et al., 2017). A recent study in dogs has suggested that radiotherapy, with or without adjunctive chemotherapy and nonsteroidal anti-inflammatory drugs, presumably achieves locoregional tumour control and prolonged survival in genitourinary carcinoma, including bladder carcinoma (Clerc-Renaud et al., 2021). However, information about a radiation protocol for the treatment of canine urinary bladder SCC is still limited. Treatment with chemotherapy in bladder carcinoma should be used in dogs with metastases. Due to the presence of metastasis in liver and abdominal lymph node, low-dose metronomic chemotherapy was prescribed in this case postoperatively. Although there is a lack of evidence for chemotherapy in dogs with urinary bladder SCC, the metronomic administration of chlorambucil in canine TCC has been shown to be well tolerated, with 70% of cases achieving partial remission or stable condition (Schrempp et al., 2013).

Conclusions

This report has described a dog that had a urinary obstruction caused by urinary bladder SCC, which occurs rarely. Partial cystectomy and ureteroneocystostomy are optional surgical techniques for SCC in the dog's ureteral orifice. Interestingly, the biomarker approach for diagnosing urinary bladder SCC in dogs should be investigated in further studies.

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