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Hematological, cytomorphological and histopathological diagnosis of transitional cell carcinoma in a male dog

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Abstract
An 8-year-old male German Shepherd dog with a history of hematuria, pollakiuria, stranguria and loss of appetite was tentatively diagnosed with urinary bladder neoplasia following hematology, urinalysis, cytology, normal abdominal radiographs and negative urine culture & sensitivity results. Abdominal ultrasound revealed a mass in the urinary bladder with considerable wall thickening and lowered treatment response to combination of chemotherapy and non-steroid anti-inflammatory drugs *viz*., vincristine and piroxicam respectively. The case confirmed to be a high grade transitional cell carcinoma by histopathology following euthanasia due to the poor prognosis.

Keywords: Hematuria, high grade, piroxicam, vincristine, urothelial carcinoma

Introduction
Transitional cell carcinoma (TCC) is the most common type of canine bladder tumor characterized by abnormal proliferation of urothelial cells. It accounts for 1-2% of all canine tumors or less than 2% of all malignant canine tumors or 50-70% of bladder tumors in dogs [1, 2]. It is considered to be a frustrating disease due to the pronounced aggressiveness or invasiveness and the lack of effective treatment both surgically and medically. These tumours commonly occur in trigone region of the bladder and may also occur multifocally due to intra-vesicle seeding. Consequently as the tumor progresses, it may eventually lead to partial or complete obstruction of the urinary tract. TCC is less commonly seen in males due to frequent urination for territorial marking, thus reducing the exposure of bladder epithelium to potential carcinogen within the urine [3]. It typically affects older animals and the mean age at diagnosis has been reported to be 11.5 years [4]. The most common manifestation of TCC are stranguria, hematuria and pollakiuria, with other possible clinical signs such as tenesmus, lethargy, lameness and weight loss with duration of weeks to months for overt manifestation of clinical signs [5].

Diagnosis of the condition may be achieved from combination of tests such as clinical signs, physical examination, urinalysis, contrast cystography, ultrasonography, cytology etc. Urine cytology or cytomorphology remains as an important non-invasive tool for diagnosis of urothelial carcinoma, despite the advent of newer techniques. The cytological features in TCC cases were increased cellularity and cell clustering, papillae, necrosis, apoptosis, high nuclear and cytoplasmic (N:C) ratio, nuclear pleomorphism, irregular nuclear margin, hyperchromasia and chromatin abnormalities [6]. However, definitive diagnosis may be achieved by histopathological examination of the bladder tissue collected through cystotomy, cystoscopy or catheter biopsy [3]. Histopathology provides a definitive diagnosis of TCC and the characterization of the different pathological types [7]. In most cases, the treatment is palliative due to the progressive nature of this disease, relative resistance to chemotherapy and difficult surgical excision due to their anatomical location [5]. Approximately 10% of dogs with TCC may develop complete urinary tract obstruction due to tumor progression and the cause of death in 60% of cases is related to the local effects of the primary tumor [4]. The consequence of the local or infiltrating disease leading to substantial impairment of quality of life is the primary reason for euthanasia in most of the canine TCC cases [8]. The present paper reports a case of Transitional cell carcinoma in a dog presented to District Veterinary Centre, Kannur with history of hematuria, pollakiuria and stranguria.

Case history and Observation

An eight year old male German Shepherd dog (Fig. 1A) was presented to District Veterinary Centre, Kannur (dist), Kerala with complaints of hematuria, pollakiuria, stranguria and loss of appetite since two weeks. Physical examination revealed mildly dehydrated animal with enlarged and painful bladder. Per-rectal examination of prostate was unremarkable. A lateral survey radiograph of the abdomen revealed no uroliths or other abnormalities. The ultrasound image revealed a heterogenous echo pattern, thickened bladder wall and irregular masses within the lumen of urinary bladder (Fig. 1B). Urinalysis of catheterized sample revealed reddish to dark brown urine (Fig. 1C) containing numerous red blood cells (Fig. 1D), 5 to 10 transitional epithelial cells with clumps in high power fields (Fig. 1E), pH of 7.0, proteinuria (3+), specific gravity of 1.027 and absence of pus cells, bacteria, casts and crystals. Urine culture and sensitivity test revealed uneventful result. Serum biochemistry revealed mild renal azotemia and complete blood count revealed mild anemia, mild neutrophilia and monocytosis on day one of examination as elaborated in Table 1.

A urinary catheter was placed to collect urine from the urinary bladder for cytological examination. Smears of the urine sediment after cytospin were stained by Giemsa staining for cytological examination. Increased cellularity and clustering of atypical transitional cells were observed in multiple smears prepared from catheterized urine sediment. The cytological features observed in the study are described in Fig 2 A-F.

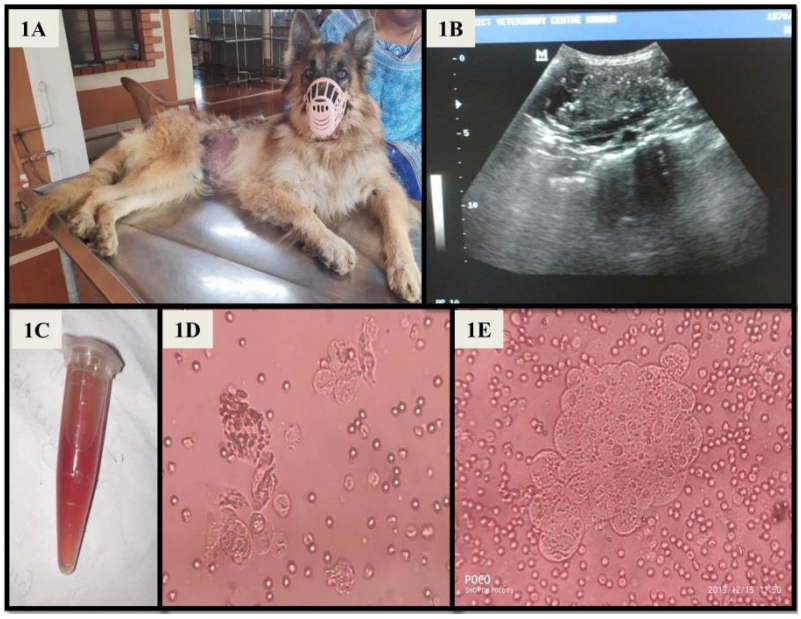

Fig 1: A. The male German Shepherd dog presented to District Veterinary Centre, Kannur (dist), Kerala; B. Heterogenous echo pattern, thickened bladder wall and irregular masses within the lumen of urinary bladder on ultrasonography; C. Reddish urine on physical examination; D. Numerous RBCs and epithelial cells on microscopic examination of urine wet mount (20X magnification); E. Transitional epithelial cells with clumps in urine wet mount (80X magnification)

Table 1: Hematological and kidney function test results of the dog

Parameters	Observed values (Day 1)	Observed values (Day 10)	Reference range	Key findings
Haemoglobin (g/dl)	11.5	10.4	12-18	Anaemia
PCV (%)	33.12	30.8	37-55	
RBC count (millions/cu.mm)	5.32	4.71	5.5-8.5	
MCV (fl)	62.26	65.39	60-77	
MCH (pg)	21.62	22.08	21-26	
MCHC (g/dl)	34.72	33.77	32-36	
WBC count (thousands/cu.mm)	17.5	20.8	6.0-17.0	
Neutrophil (thousands/cu.mm)	13.82	17.8	3-11.5	
Lymphocytes (thousands/cu.mm)	1.93	1.46	1-4.8	
Monocyte (thousands/cu.mm)	1.75	1.6	0.15-1.350	
Neutrophil (%)	79	85	58-85	
Lymphocytes (%)	11	7	8-21	
Monocyte (%)	10	8	2-10	
Eosinophil	0	0	0-9	
Platelet (lakhs/cu.mm)	2.47	0.53	2-5	
MPV (fl)	7.0	7.9	6.7-11.1	
Creatinine (mg/dl)	1.58	3.35	0.5-1.5	
Urea (mg/dl)	89.12	120.5	10-28	

The case was tentatively diagnosed as transitional cell carcinoma. Vincristine sulfate @ 0.025 mg/kg and Piroxicam @ 0.3 mg/kg intravenously and other supportives were administered with frequent monitoring of the animal. However 10 days later, the owner reported worsening of condition of the dog with dark urine, weakness and recumbency. CBC and serum biochemistry (in Table 1) along with persistence of the mass on ultrasonography revealed

deterioration of body condition. Based on the poor prognosis, a sensible decision of humane euthanasia was opted as per the request of the owner. A detailed postmortem examination was conducted during which the entire urinary bladder and sections of kidney were collected and sent to Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, KVASU, Lakkidi, P.O., Wayanad District-Kerala, India. On gross examination, congested, enlarged and diffusely thickened urinary bladder with multiple cauliflower like sessile masses on the mucosa projecting in to the lumen was noted as in Fig 2G. The right kidney was severely deformed as shown in Fig 2H. The cortex of right kidney was atrophic and shrunken and the pelvis was dilated.

On histopathological examination, plaque like growth pattern on the mucosal surface could be observed with nesting of tumour cells; and invasion deep into submucosa and muscularis propria of the bladder wall. The tumor cells were atypical characterized by loss of polarity, pleomorphism, hyperchromatic nuclei, and frequent mitoses. The nuclear contour was irregular and distinct nucleoli could be observed in cells.

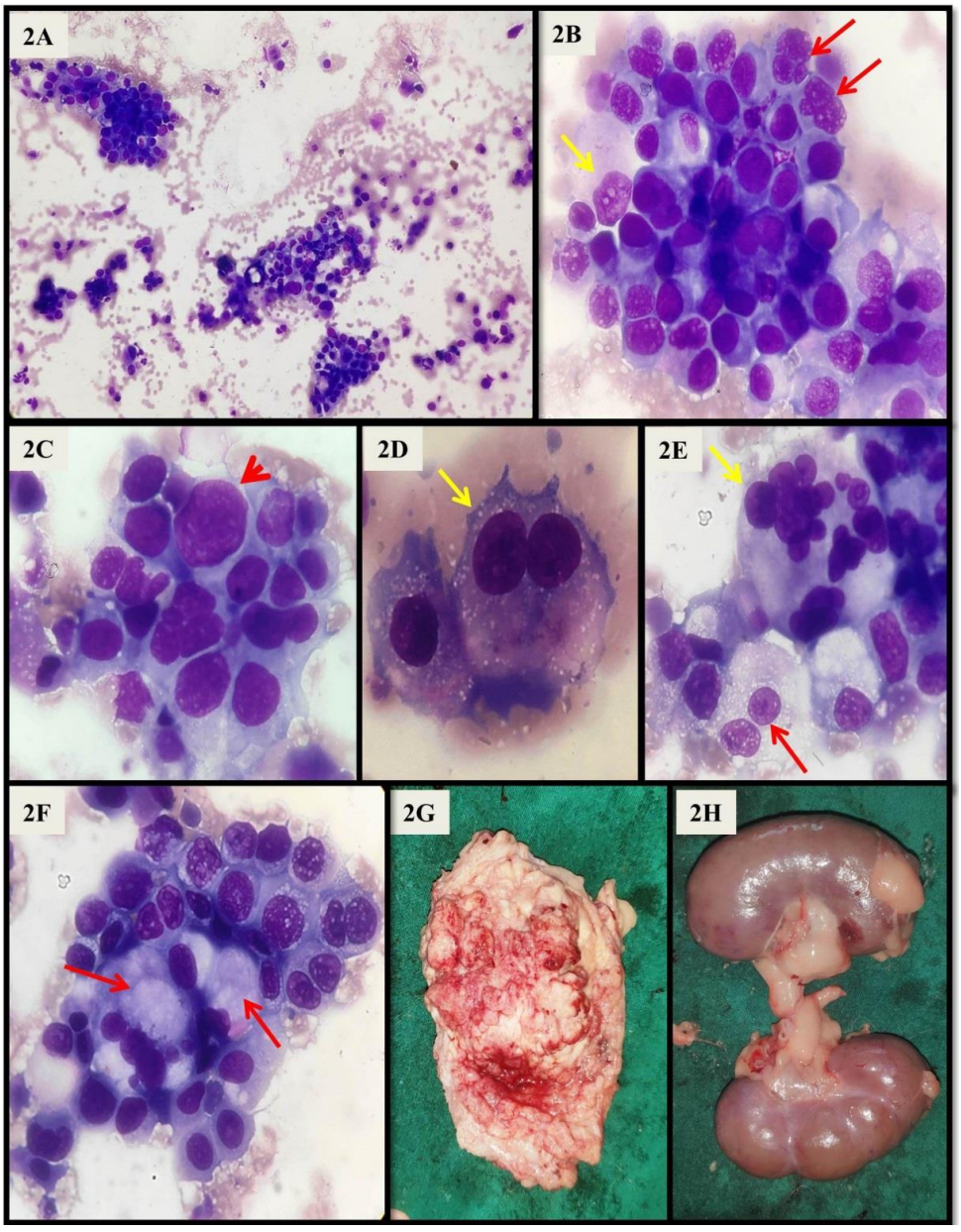

Fig 2: A. Small to large sized clusters or islands of aggregated carcinoma cells (Giemsa staining; 10X magnification); B. Sheets of cohesive atypical cells with high nuclear: cytoplasmic (N:C) ratios containing round to slightly oval often eccentrically located nucleus with irregular borders, sometimes binucleated or multinucleated cells (red arrow), multiple prominent nucleoli (yellow arrow) and mildly clumped to lacy chromatin (Giemsa staining; 200X magnification); C. Clusters of cells with round to polygonal shape, basophilic cytoplasm, high N:C ratio, anisokaryosis, anisocytosis and megakaryocyte or macronuclei (red arrowhead) (Giemsa staining; 200X magnification); D. Cells resembling umbrella cells with uninucleated to binucleated nature (yellow arrow) with abundant amount of amphophilic cytoplasm with numerous vacuolations and irregular cell borders or cytoplasmic tails (Giemsa staining; 200X magnification); E. Tumor giant cells (yellow arrow) with relatively abundant foamy cytoplasm (red arrow), multiple nuclei, nuclear pleomorphism, hyperchromasia, irregular nuclear membranes, nuclear molding or nuclear overlap (Giemsa staining; 200X magnification); F. Large cytoplasmic vacuoles (red arrow) pushing nucleus to the periphery and reticular chromatin pattern. These cytological features were compatible with malignant cells (Giemsa staining; 200X magnification); G. Grossly the urinary bladder wall was diffusely thickened with multiple cauliflower-like sessile masses on the mucosa projecting in to the lumen; H. The cortex of right kidney was atrophic and shrunken and the pelvis was dilated.

Discussion

Transitional cell carcinoma (TCC), also known as urothelial carcinoma, is considered to be a high grade invasive cancer causing urinary tract obstruction, distant metastases in more than 50% of affected dogs affecting the quality of life of the animal. The probable risk factors are insecticides or ectoparasiticides, obesity, female animals and a very strong breed associated risk [9]. The clinical signs like hematuria, stranguria and pollakiurea in TCC closely mimic those of a urinary tract infection like chronic cystitis, where urinalysis and ultrasonography comes into importance. In the present case, urinalysis ruled out cystitis due to normal urine pH and absence of pus cells and bacteria. In addition, urine culture at initial stage of presentation aided in more rapid diagnosis of the tumorous condition by ruling out bacterial infection. The cytospin smears were highly cellular with clusters of urothelial cell and occasional single cells. The cells were oval to polygonal with pleomorphic hyperchromatic nuclei with coarse chromatin, prominent single to multiple nucleoli and irregular nuclear membrane. The cytoplasm of these cells were scant to abundant with vacuolations, mild to moderate basophilia and occasionally amphopathic. In certain areas, large cytoplasmic vacuolations were observed pushing the nuclei to the periphery which might be due to the presence of mucus within the cells.

Upon these criteria, our cytopathological observation including increased N:C, cytoplasmic basophilia, cytoplasmic vacuolation, irregular nuclear borders, nuclear molding, anisocytosis, anisonucleosis, karyomegaly, hyperchromasia, prominent or multiple nucleoli, coarse or ropy chromatin, cell clusters and multi nucleated umbrella cells are compatible with high grade urothelial carcinoma[1]. Similar malignant features of high N:C, hyperchromasia, chromatin alterations, nuclear pleomorphism and irregular nuclear margins were most frequently observed in malignant atypical cells [6]. However, they also pointed that increased cellularity and cell clustering had been noted in many benign urine samples due to the fact that exfoliation of transitional cells occurs in many disease conditions like urolithiasis, cystitis, viral infections, chemotherapy and benign prostatic hyperplasia. Urinary cytology is a simple, cheap and noninvasive method however, with variable sensitivity and accuracy due to several factors like improper sampling and inadequate cellularity, cellular degeneration due to overnight stagnation in bladder, inadequate cellular exfoliation in low grade carcinoma and also due to lack of universal standardized reporting criteria [10]. The diagnostic criteria to differentiate bladder carcinomas and reactive urothelial cells are nuclear hyperchromatism and pleomorphism than nuclear or cell size. In lowest grade lesions, nuclear crowding is the first clue that the epithelium is abnormal and thus individual cells within groups should be examined in detailed for diagnostic criteria in bladder washing specimens [11]. It is also stated that malignant cells resembling umbrella cells can be identified on urine cytology in up to 17% of cases of high grade bladder carcinoma identified by presence of elevated N/C ratio, prominent nucleoli, or obviously malignant nuclei [12].

It has been previously documented that ultrasound is a valuable non-invasive first-line reproducible imaging modality for detecting tumors that infiltrate the muscle layer of the bladder wall [13]. The main diagnostic features of tumour infiltration in bladder wall was interruption of hyperechoic (submucosal) and hypoechoic (muscular) layers in the site of the lesion as previously described [14]. On ultrasonography, heterogeneous echogenicity, bladder wall involvement, broad based attachment and progressive tumour size evaluation on piroxicam therapy could be helpful in both diagnosing and predicting prognosis in canine TCC [15]. Ultrasound was the imaging modality used in this case to confirm the presence of the abnormal mass within the bladder and the unusual wide thickening of the wall suggestive of TCC.

Histopathology is considered as final diagnostic test to confirm the malignancy in urothelial neoplasms. Presence of invasion into submucosa and muscularis mucosa along with pleomorphism of cells, mitosis and nuclear abnormalities in this case confirm the condition as invasive or high grade transitional cell carcinoma. In 2016, WHO classification of tumors of the urothelial tract categorizes papillary urothelial neoplasms into four groups: urothelial papilloma, papillary urothelial neoplasm of low malignant potential (PUNLMP), low-grade papillary urothelial carcinoma (LGPUC), and high grade papillary urothelial carcinoma [16]. Based on another classification by WHO, 78% of dogs diagnosed with TCC are in T2 stage indicating invasion in bladder wall and 20% at T3 stage indicating invasion into adjacent viscera like prostate uterus, vagina and pelvic canal [7, 9].

The treatments for TCC in dogs reported to be are surgery, medical therapy, radiation therapy and local intravesical therapy. Potential indications for surgery are procurement of biopsy samples, excision of tumour or maintenance of urine flow. However, surgical intervention are limited in TCC cases due to typical trigonal location, urethral involvement, metastatic stage at diagnosis and post-surgical diffuse malignant urothelial transformation (field effect) [4, 9]. In general, canine TCC is considered relatively resistant to chemotherapy. However, systemic medical therapies with chemotherapy agents and COX inhibitors (NSAIDs) are included in the mainstay treatments. In addition, efficacy of multiple chemotherapeutic protocols and drugs like cisplatin, carboplatin, mitoxantrone, doxorubicin, vinblastine, and gemcitabine have been studied [16]. The antitumour mechanism of NSAIDs is not completely understood, but it was found that COX-2 inhibitor like piroxicam, meloxicam, Deracoxib and firocoxib can induce tumour apoptosis, anti-angiogenesis and reduced basic fibroblast growth factor concentration [17]. Radiation therapy has been performed infrequently in dogs, the use of palliative external beam radiation therapy in conjunction with mitoxantrone and piroxicam has been reported with considerable clinical improvement [18]. Other treatment modalities for canine TCC are emerging, and these include metronomic chemotherapy, folate-targeted therapy, and demethylating agents [9].These carcinomas in general carry a bad prognosis, with a reported median survival period with combined treatment modalities ranging from five to twenty three months[2]. In present study, vincristine in combination with piroxicam was used as it is previously found that nonselective COX inhibitor, piroxicam, enhances the activity of vinblastine in naturally occurring canine model of invasive urothelial carcinoma [19]. In conclusion, it can be stated that this case showed the value of urine cytology and ultrasound examination in making a tentative diagnosis of TCC and the gold standard test of histopathology for confirmatory diagnosis. Treatment modalities like chemotherapeutic drugs or use of the NSAID piroxicam would have benefited if the animal was presented at an earlier stage and probably prolonged this dog's survival time.

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