

## Investigation of Clinical, Radiography and Ultrasound, Biochemical, Cytological and Histopathological Findings of Anal Sac Adenocarcinoma in Spitz Dog

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**Abstract:** A 9- year -old, 7kg emasculated male white Spitz dog was referred to small animal hospital of the Tehran Veterinary College for evaluation of a mass associated with the left anal sac. On digital rectal examination, a mass little stiff left anal sac, that was of  $3.2 \times 2.8 \times 1/0$  cm in size, approximately rigid, swelling in ventrolateral regions. Additionally, enlarged regional lymph node was noted on abdominal palpation. Abdominal ultrasound suggested this structure was iliac lymph node, ranging in size from 2.4 cm to 3.7 cm. The mass was excised and a histological diagnosis of ASAC was made. No evidence of metastasis on thoracic radiographs or abdominal ultrasound was seen. Laboratory data biochemical profile demonstrated, Azotemia and hypercalcemia that indicates an elevated total calcium of 16.3 mg/dL (reference ranges, 8.2–12.4 mg/dL or 9.3-11.4mg/dL) and hypophosphatemia (mean = 3.4 mg/dL, reference ranges: 2.5\_5.6 mg/dL),also increased enzyme levels ALP, AST, ALT were present, Results indicated, following surgery, signs hypocalcemia was noted. Repeated ionized calcium mensuration confirmed hypocalcemia and hypercalcemia of malignancy panels proposed parathyroid gland suppression as the cause. Clinical signs related either to regional iliac lymph node large (tenesmus) or hypercalcemia (polyuria-polydipsia, lethargy and weakness). Cellular features were with cumulative of epithelial cells with nuclear of coarse chromatin pattern and abundant basophilic cytoplasm. Histopathology showed a well demarcated expansile neoplastic proliferation of large cuboidal or polyhedral cells forming multiple tubules and acini in a coarse desmoplastic stroma with the presence of inflammatory cells.

**Key words:** Surgery • Dog • Histopathology • Tumor • Hypercalcemia

### INTRODUCTION

Skin and soft tissues have a wide range of tumors and many of them are of most common neoplasms in veterinary medicine. Skin's lesions and masses are easily recognized by the owner or the veterinarian [1]. The anal sacs are small paired organs located near the anus between the external and internal sphincter muscles [2, 3, 4]. Anal sac adenocarcinoma (ASAC) is a malignant tumor which originates from apocrine glands of the anal sac [1, 2, 5, 6, 7]. Meuten *et al.*, (2002). Categorized the tumors into 3 types as solid, tubular and rosette [1]. Ogawa *et al.*, (1999). Reported the fourth type as a papillary [8]. ASAC is reported to demonstrate 2% of all

skin tumors and 17% of anal sac neoplasm's in dogs, despite it is an unusual neoplasm, it is locally invasive and has a high acceleration of metastasis to the draining iliac lymph nodes [9-12]. Anal sac adenocarcinomas can cause serious problems, because they have a tendency to metastasize to nearby tissues and organs, usually metastasize to sacral, iliac and lumbar lymph nodes by lymphatic drainage and metastasize less to liver, lungs and spleen [9, 13, 14, 15, 16]. In a study, Bennett *et al.*, (2002) indicated that (53%) dogs had hypocalcaemia and (79%) had metastases [13]. Malignant neoplasms that are usually related with humoral hypercalcemia of malignancy (HHM) in animals are: the Adenocarcinoma originating from apocrine glands of the anal sac in dogs, some Tcell

lymphomas in dogs, myelomas and miscellaneous carcinomas [17, 18]. Inordinate secretion of PTHrp is the main reason of hypercalcemia in most forms of HHM [19]. Anal sac tumors are most commonly seen in neutered female and castrated male dogs [2, 10, 14, 16, 20, 21, 22, 23]. The affected dogs are usually between 5 and 15 years -old [1, 14, 10, 21, 22] and most commonly affected breeds are Husky, Siberian, Bull dog and Alaskan malamute [3, 4, 21, 24]. ASAC is usually unilateral [14] and is known as hepatoid tumor because its cells look like hepatocytes [14]. Between 25-50% of dogs with ASAC will have hypocalcaemia as well [2, 23, 25, 26]. We don't know exactly what the causes these to occur, any dog can develop ASAC but some breeds are more susceptible, malignant tumors are widely seen in older dogs [27, 28]. The purpose of the investigation is to describe Clinical signs, Radiology and Ultrasonography, Clinical pathology and Pathological Finding of ASAC for the first time in Iran.

## MATERIALS AND METHODS

In July 2011. A 9- year -old, 7kg, castrated male white Spitz dog with a mass, sized: 3.2 cm × 2.8 cm × 1/0 cm around anus was referred to small animal hospital of Tehran veterinary faculty. At first this case was referred for clinical examinations, after clinical examinations of the area, the case was referred to Radiology and Ultrasonography department to find out whether the mass had metastasis to the regional lymph node, abdominal area and further areas such as lungs and to survey whether it is malignant or benign, then the mass was biopsied using fine-needle aspiration method and the following steps were taken:

**Fine Needle Aspiration Biopsy (FNAB):** To collect fine needle aspirate, the effected anal sac gland was held firmly in position with free hand and the perforation site was cleaned with alcohol, A 22 gauze small needle attached with 5 ml syringe was inserted in to the mass then to the iliac lymph node separately. A strong negative pressure was applied by exhaust the plunger. The needle was moved through the mass three or four times in different directions. Still with the needle in mass, suction was slowly released. This procedure was repeated from multiple sites of effected gland. Then aspirated the cellular material from the syringe into the needle, forced this material onto a glass slide and spread it into a thin film.

Then the slide was stained in order to identify the cells within the sample. Then referred the slide to a clinical pathologist for cytologic evaluation.

**Urine Sampling:** Before catheterization the dog required sedation. We passed a catheter gently from urethra to the urinary bladder and collected the urine in a sterile universal container and then the urine sample was referred for analysis.

**Blood Sampling:** We used cephalic vein to take a blood sample. Muzzled the dog and tied a tourniquet right above his elbow so that the cephalic vein would fill with blood. After disinfection of the area with alcohol, 10cc of blood was taken with a syringe and then the area was again disinfected with alcohol. The sample was sent for analysis. The samples were prepared in two parts, the first one was a vial containing 50microliters of anticoagulant (EDTA) and 3ml of blood for hematology examinations and the second was a test tube with no anticoagulant, containing 5-7ml of blood for separation of serum and biochemical examinations.

**Surgery Technique (Closed Technique):** At first palpated the anal sacs to determine their location and extent by placing the middle finger in the rectum and thumb over the sac and evacuated the feces from the rectum. Then prepared the preanal area for surgery. After injected Atropine (0/02-0/04mg/kg. IV) as premedication, Thiopental (10-12mg/kg. IV) as induction and Isoflurane as maintenance. First inserted a balloon-tip catheter into the orifice of the anal sac duct, after dissected against the anal sac through a curvilinear incision. And then separated the sac's outer part from the internal, external and sphincter muscles surrounding it using a metzenbum. Also placed a ligature around the duct at the mucocutaneous junction with a 4-0 polydioxonore polyglyconate, in the last excised the anal sac and duct and closed the skin with 0-4 nylon sutures. Fig. 1: Schematic diagram demonstrating the surgical incision locations for closed and open anal saccullectomy techniques (taken from surgical views).

**Touch Imprint Smear Method:** After surgery was cut the mass into pieces using a sterile scalpel, put some 50mm pieces in formalin (10%) for pathological studies, then put a small piece of the mass on a glass slide and pressed it properly in order to prepare a smear. After used

White-Giemsa method for staining (20 minutes). At first the glass slide was fixed in alcohol (ethanol 96%) for 20 seconds and after dried, at this time cytologic evaluation was performed.

**Histopathological Study:** After cytologic studies. Tissue specimens were fixed in 10% formalin, processed routinely, embedded on paraffin, sectioned in 5 $\mu$  and stained with haematoxylin-eosin (H&E) for microscopic examination of histopathology.

## RESULTS

**Clinical Finding:** The anal sac mass was approximately firm, swelling in ventrolateral. The 3.2 cm  $\times$  2.8 cm  $\times$  1/0 cm sized. Signs included polyuria, polydipsa, lethargy, weakness that are related to hypercalcemia, local irritation (which may result in bleeding and licking of the anus), tenesmus or constipation associated with enlargement of the iliac lymph node.

**Cytological Findings:** Smears made of needle aspirates from a mass located in the region of the left anal sac gland and touch imprint after surgical removal, for cytology examinations. The smears contain many large clusters of epithelial cells. Cytology evaluation can be practical in the clinical diagnosis of neoplasia. Cytologically, touch imprint smear of adenocarcinomas were characterized by anisocytosis and anisokaryosis, hypercellularity, increased nuclear to cytoplasmic ratio and prominent nucleoli round, shaped cells were observed which resembled hepatocytes and high number of cellular sheets, a mass of epithelial cells having a nucleus of coarse chromatin pattern, plenty of basophilic cytoplasm and Cytoplasm vacuolation. Perianal adenocarcinoma is difficult to differentiate from adenoma cytologically.

**Ultrasound Findings:** There was a mass in ventro-lateral region with heterogenous echo. Also a cystic region on cranial part of the tail with a high vascularization pattern, dog had iliac lymphadenopathy on abdominal ultrasonographic examination (enlargement about 2.4-3.7 cm). No evidence of metastasis to other organs was noted with imaging.

**Radiography Findings:** No evidence of pulmonary metastasis was noted on thoracic radiographs. Mild signs of bronchial pattern was seen which could be considered

as a normal finding in this age. Also investigation spinal radiographs of the thoracolumbar region taken under general anesthesia, did not show any evidence of metastatic.

**Histopathological Findings:** The most important feature in histopathology findings was the invasion of tumor cells into the connective tissue which is an indicator of malignancy. The neoplastic cells are polygonal in shape with a light pink (clear) cytoplasm with round distinct nucleus and clear nucleolus, shaped lobular pattern that is supported by a fine fibrovascular stroma. The reserve cells showed pleomorphism and hyperchromatic nuclei. Forming multiple tubules and acini in a coarse desmoplastic stroma. Present inflammatory cells may mask the presence of neoplastic cells. Inflammation was characterized by a mixed population of cells including neutrophils and lymphocytes, in some areas. These inflammatory reactions often result in hyperplasia of surrounding tissues. The hepatoid cells had a vacuolated cytoplasm, large nucleus with several prominent nuclei, white-eosinophilic cytoplasm with the mitotic rare figures.

**Care:** Removal of tumor with surgical was treated. No dog was administered chemotherapy or radiation therapy, Of course calcium concentrations and clinical signs were controlled with prednisone.

## DISCUSSION

Clinical, gross, cytologic, radiographic, ultrasound, blood and serum tests and light microscopic findings are described for this male dog. With adenocarcinomas arising from the apocrine glands of the anal sac, the tumor had light microscopic features of malignancy and abdominal ultrasound indicated enlargement iliac lymph nodes. ASAC in dogs is one of the most rare types of cancers [3, 14, 19, 22] that is a malignant lesion in dogs; it develops from the apocrine secretory epithelium located in the wall of the anal sac [3, 21] can also originate from the preanal area [3, 14, 29]. Macroscopic results in this study demonstrated that tumor develop as a approximately firm mass in the perianal area, swelling in ventrolateral regions, in close association with the anal sac that arises in the wall of the anal sac. It usually occurs in the average age of 10 years [24]. Over 50% of cases have regional nodal invasions at the time of diagnosis [24]. A multimodal therapeutic approach combining

surgery, radiation therapy and chemotherapy is often necessary for prolonged disease control [13, 20, 26, 30]. Persistent hypercalcemia (91%) and hypophosphatemia (71%) are two characteristics of this syndrome [24]. Malignant neoplasms that are usually related with humoral hypercalcemia of malignancy (HHM) in animals are: the Adenocarcinoma originating from apocrine glands of the anal sac in dogs, some Tcell lymphomas in dogs, myelomas and miscellaneous carcinomas. Inordinate secretion of PTHrp is the main reason of hypercalcemia in most forms of HHM [17, 18]. Hypercalcemia usually is related with this particular adenocarcinoma. A number of mechanisms can be assumed to describe the constant hypercalcemia in association with tumor [25, 31]. The findings in this survey evidence a unique clinicopathologic syndrome characterized by ongoing hypercalcemia and hypophosphatemia associated with an adenocarcinoma originated from apocrine glands of the anal sac, in this study results showed hypercalcemia (16.3 mg/dl) and hypophosphatemia (3.4 mg/dl). Adenocarcinoma causes an increase in serumcalcium in three ways: 1. humoral hypercalcemia of malignancy 2. hypercalcemia which is caused by metastases of solid tumors to bone 3. Hematologic malignancies [17] and lead to a toxic effect on normal tubules in two ways, direct and the other way is by ischemia which is caused by vasoconstriction. Severe or long hypercalcemia can lead to renal failure [32] has multiple effects on kidney

function. First, the collecting duct response to ADH is diminished leading to polyuria and polydypsia, hyponatremia and decreased urine specific gravity. That assumed to be caused by decreased cAMP concentration in collecting duct epithelial cells secondary to elevated serum calcium concentrations. Glomerular filtration rate also is decreased due to calcium-induced vasoconstriction of the renal vasculature [33]. The attendance of a humoral substance secreted by ASAC is strongly suggested by the rapid decrease of serum calcium concentrations after resection of the neoplasia and the after return of hypercalcemia with tumor recurrence [34]. Sevil Atalay Vural *et al.*, (2005) worked on Anal sac carcinoma in dogs, in their study dogs showed persistent hypercalcemia and hypophosphatemia [35]. The loss of primary renal lesions or significantly increased blood urea nitrogen(BUN) (A biochemical profile indicates an elevated BUN of 120 mg/dL) and creatinine (2 mg/dL) values, together with atrophic parathyroid glands, rules out secondary renal hyperparathyroidism or primary hyperparathyroidism as the mechanism responsible for the hypercalcemia in this dog. In this study clinical and historical signs were related to enlargement of iliac lymph nodes and hypercalcemia which was observed for approximately 1-2 weeks. Signs included: polyuria and polydypsia associated with hypercalcemia, tenesmus or constipation related to enlargement of regional lymph nodes.

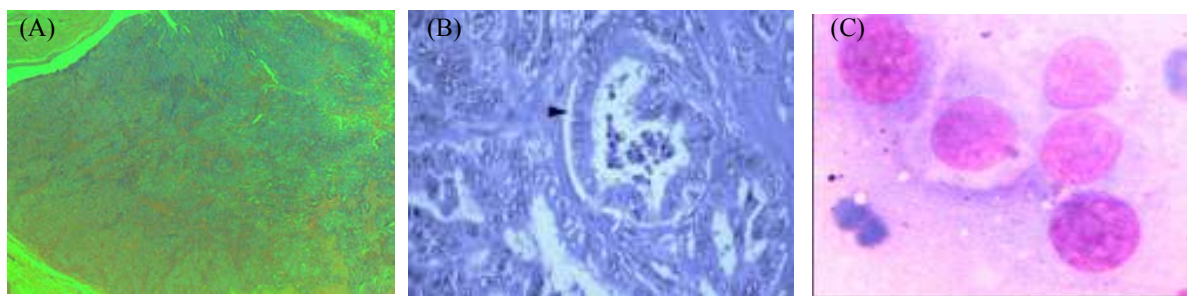


Fig. 1: Histopathology indicated that **A**: small groups of proliferating cells invading the stroma were identified and there was stromal haemorrhage and well as the presence of inflammatory cells including neutrophils and lymphocytes. The subepithelial connective tissue is expanded by an unencapsulated, multilobulated and invaded to the surrounding tissue glands and nearby tissues lobular pattern that is supported by a fine fibrovascular stroma. **B**: showing irregular acini containing degenerate cellular debris (arrowhead) and lined by a cuboidal epithelium exhibiting marked cellular pleomorphism (H&E), Polygonal cells with a light pink (clear) cytoplasm with round distinct nucleus and clear nucleolus. Cells resembled hepatocytes and pleomorphism **C**: Cytology on an anal sac adenocarcinoma at 500x. note that the Cumulative epithelial cells with nuclear of coarse chromatin pattern and abundant basophilic cytoplasm, anisokaryosis, anisocytosis and Cytoplasm vacuolation

Table 1: Serum biochemical parameters of a spitz dog with ASAC

	Result	Normal		Result	Normal
Color	Yellow		ALP(V/L)	184 H	(90-150)
Appearance	Clear		AST/GOT(V/L)	80 H	(5-55)
Specific Gravity	1/005		ALT/GPT(V/L)	122 H	(5-60)
PH	8/0		Ca(mg/dl)	16.3 H	(9.4-11.6)
Protein	2+		P(mg/dl)	3.4 L	(2.5-5.6)
RBC	0-1				
WBC	2-3				
Epithelial Cells	Many				
Bacteria	Many				
Total Protein (TP)	7.5	(5.1-7.8)			
Albumin (g/dl)	2.6	(2.6-4.3)			
Globulin (g/dl)	3.5	(2.3-4.5)			
Glucose (mg/dl)	83	(60-125)			
Triglycerides (mg/dl)	103	(20-150)			
Cholesterol (mg/dl)	212	(129-330)			
BUN (mg/dl)	120 H	(8-29)			
Creatinine (mg/dl)	2 H	(.4-1.8)			
Urea (mg/dl)	60.7	(35-70)			

ALT: alanine aminotransferase, AST: aspartate aminotransferase ALP: Alkaline phosphatase

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