



TUBULAR INTESTINAL ADENOCARCINOMA IN A CAT: PATHOMORPHOLOGICAL AND IMMUNOHISTOCHEMICAL FINDINGS

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Summary

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Intestinal adenocarcinoma is a malignant tumour originating from the glandular intestinal epithelium. The tumour is relatively uncommon in cats. The most affected site in cats is the ileum when compared to other species. In the case, tubular type adenocarcinoma was diagnosed in the caudal part of duodenum of 1.5-year-old female Siamese cat. Two masses of different sizes were detected on the duodenum, protruding and obstructing the lumen. Histopathologically, anaplastic glandular epithelia tried to form tubules. The anaplastic cells invaded into tunica muscularis. Mononuclear cell infiltrations were present in all layers of the duodenum. The masses were evaluated immunohistochemically by the strept ABC method and different markers (Ki67, Her2neu, CEA and p63). The first three markers were positive in anaplastic glandular epithelial nuclei, cytoplasm and membrane in the propria of the mucosa and submucosa. Ki67 and Her2neu in particular, predicted the high malignancy of anaplastic cells and poor prognosis of tumour.

Key words: feline tubular adenocarcinoma, gut, markers, pathomorphology

Gastrointestinal tumours constitute 1% of all tumours in cats and dogs. Their occurrence in small intestine is lower than in the large intestine in terms of adenocarcinoma (Cribb, 1988; Leib & Matz, 1995; Risetto *et al.*, 2011; Blois, 2012). The neoplasm is generally localised in the jejunum and the ileum (Head *et al.*, 2002). Older animals are more predisposed to the tumour and it is seen at an average age of 11–12 years. It is more frequently encoun-

tered in Siamese cats although a gender predisposition was not reported. Its predilection sites are the small intestine in cats, while in dogs the colon and the rectum are most commonly affected (Kosovsky *et al.*, 1988; Burrows *et al.*, 1995; Leib & Matz, 1995; Blois, 2012). The adenocarcinoma develops generally as local and invasive, but grows slowly. It has a good prognosis, but sometimes can metastasise to lymph nodes and/or adhere to the perito-

neum when it has highly malignant features (Twedt, 1981; Blois, 2012). In such instances the prognosis is poor even though it is excised surgically because of its recurrence risk (Kosovsky, 1988; Ogilive, 1995; Bedford, 1998).

Most common clinical findings are depression, anorexia, distinct weight loss for 5 to 6 months, emesis, abdominal tension due to the mass and ascites, tenesmus and constipation (Patnaik, 1976; Blois, 2012). Macroscopically, the mass grows circularly around the intestinal wall (Cribb, 1988; Ogilive, 1995). With regard to microscopical features, 4 types are distinguished – tubular, papillar, mucinous and signet ring cell forms (Head, 1976; Head *et al.*, 2002). There are no reports related to the tubular type.

There are no previous reports using immunohistochemical markers in feline intestinal adenocarcinoma. However, canine and human intestinal tumours including adenocarcinomas are demonstrated by Ki67 and Her2, carcinoembryonic antigen (CEA), cyclooxygenase-2 (COX-2), p53, bcl-2, hepatocyte paraffin-1 and cytokeratin (Ramos-Vara & Miller, 2002; Wendum *et al.*, 2003; Park *et al.*, 2007; Menezes *et al.*, 2010, Anita *et al.*, 2012; Damasceno *et al.*, 2012). CEA has a high sensitivity for detection of early-stage colorectal cancers in humans (Fernandes & Matos, 2002). Her2 is used for detection of anaplasia in colorectal cancers of humans and dogs (Schuel *et al.*, 2006; Damasceno *et al.*, 2012). Ki67 is mentioned as

a marker defining proliferation and biological behaviour of the neoplasm (Kanasvaros *et al.*, 1999). There is no documentation about p63.

In this case, a highly malignant tubular adenocarcinoma in duodenum was evaluated by using different markers in a 1.5 years old female Siamese cat. The patient was referred to the clinics in Ankara University, Faculty of Veterinary Medicine, with complaints of anorexia, lethargy, abdominal distention and constipation. The radiography revealed a mass in the gut that was operated. The excised material including the entire duodenum was sent to the Department of Pathology for diagnosis. After macroscopical examination, tissue samples were fixed in 10% formalin. The tissue samples were processed routinely and embedded in paraffin. Then, they were stained with haematoxylin-eosin (H&E), Masson's trichrome and Mayer's mucicarmin. For immunohistochemistry, sections on adhesive slides were deparaffinised and dehydrated. Peroxidase activity was blocked in 3% hydrogen peroxide-methanol solution. Trypsinisation was performed by using 0.1% trypsin solution. Non-specific proteins were blocked by incubating protein blocking sera for 30 min at 45 °C (Peroxidase Detection System, Ready to use, RE7120-K, Novocastra). The sections were incubated with primary antibodies for 1 h at 45 °C (Table 1). Following this, biotinylated link antibody and horse radish peroxidase-streptavidin antibody were applied on

Table 1. Primary antibodies and dilutions

Primary antibody	Product brand	Dilution
Monoclonal rabbit anti p63	PIN001-0.5, Biologo	1:40
Monoclonal mouse anti CEA	CD66 Ab-3, Clone Col-1, Labvision	1:100
Monoclonal mouse anti Her2neu	NCL-CB11, Novocastra	1:40
Monoclonal rabbit anti Ki67	RM9106-S0, Clone SP-6, Labvision	1:50

sections, respectively (Peroxidase Detection System, Ready to use, RE7120-K, Novocastra). The sections were incubated for 45 min at 45 °C. As chromogen, aminoethylcarbazole (AEC) was selected (Santa Cruz). Counterstaining was performed using Gill's haematoxylin. Sections were mounted with glycergel. All sections were treated with PBS after each step. For control sections, PBS was used instead of primary antibodies. The rest of procedure was identical. Slides were examined on light microscope (Leica DM4000B) and photographed (Leica DFC280).

The excised material weighed 12 g and had a diameter of 6.5 cm. There were two masses located in the duodenum. They were found together. The first one protruded

into the lumen and the gut was enlarged. The first mass was 3×1.5×2 cm of size, grayish-white in colour and with firm consistence. It filled the lumen and its cut section had a smooth surface. The second mass was 2×1×0.5 cm of size. It had generally the same appearance, but was seen as multilobular (Fig. 1).

In histopathological examination, atypical intestinal glandular epithelial cells with small, ovoid and hyperchromatic nuclei and scanty cytoplasm were observed. The cells formed tubular structures and some of them invaded into the muscle bundles in tunica muscularis (Fig. 2 and 3). Moreover, diffuse lymphocytic infiltrations were seen in lamina propria, submucosa and tunica muscularis (Fig. 4). Masson's trichrome stain differentiated epithelial cells from connective and muscle tissues (Fig. 5). After Mayer's mucicarmine staining, cytoplasm of neoplastic glandular epithelial cells were mucin positive (Fig. 6).

Immunohistochemically, positive reactions were obtained by three markers. A mild positive reaction was detected by the Her2neu marker in highly anaplastic cells invading into tunica muscularis (Fig. 7). Slight positive reactions were obtained with the CEA marker (Fig. 8). Ki67 positivity was granular in appearance in the nuclei of anaplastic gland epithelial cells which invaded into mucosal propria and tunica muscularis (Fig. 9). No reaction was observed with the p63 protein.

It is documented that feline gastrointestinal adenocarcinomas are generally seen in elderly, male animals and Siamese (Cribb, 1988). It is mentioned that the survival time of cats diagnosed with tubular adenocarcinoma is approximately 11 months but that patients can survive up to 28 months after surgical operation (Lingeman & Garner, 1972; Cribb, 1988). In the

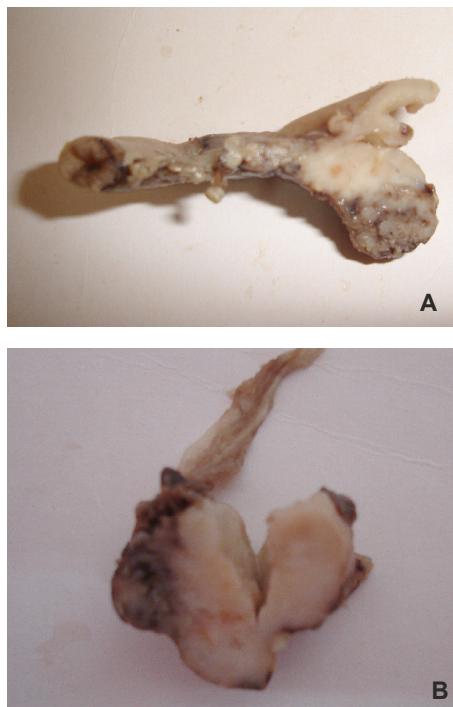


Fig. 1. Macroscopical appearance of the mass developing in the gut (A) and its cut section (B).

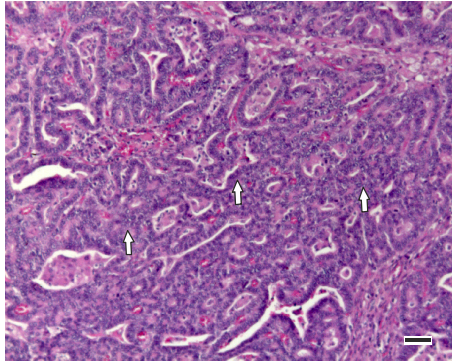


Fig. 2. Anaplastic intestinal glandular epithelia (arrows). Bar=35 μ m, H&E.

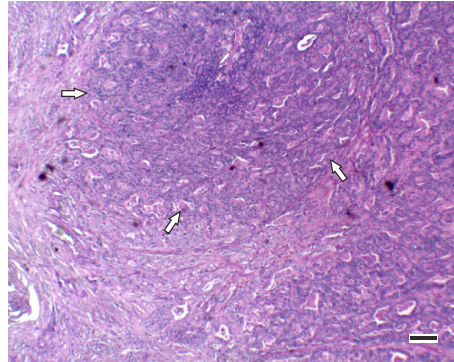


Fig. 3. Anaplastic glandular epithelia invading tunica muscularis (arrows). Bar=35 μ m, H&E.

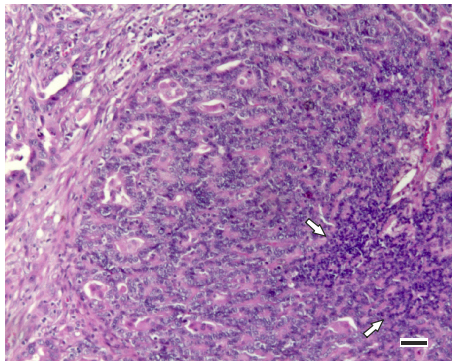


Fig. 4. Focal mononuclear cell infiltration composed of high proportion of lymphocytes (arrows). Bar=30 μ m, H&E.

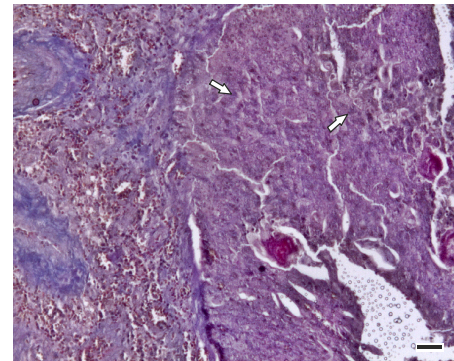


Fig. 5. Anaplastic intestinal epithelial cell islands (arrow) and stroma. Bar=35 μ m, Masson's trichrome stain.

reported case, the Siamese cat was female and 1.5 years of age. The health status of the cat was checked at regular intervals after the operation. She was still alive 15 months after the surgery despite the poor prognosis of the tumour.

Intestinal adenocarcinoma is a highly malignant tumour originating from glandular epithelium of gut (Head, 1976; Cribb, 1988). It frequently invades the wall of gut (Head, 1976). In the tubular type adenocarcinoma, the World Health Organization (WHO) has described histopathologically many tubules or tubule-like formations composed of anaplastic gland epithe-

lium. Monolayer cuboid and columnar gland epithelia sometimes constitute cell islands (Head, 1976). In the present case, these tubular formations and cell islands were composed of anaplastic cells. However, the anaplastic cells invaded into propria mucosa and tunica muscularis as in *in-situ* carcinoma reported by Shamsuddin *et al.* (1985). Additionally, there were many lymphocyte infiltrations in mucosal propria. The anaplastic cells were considered as tubular adenocarcinoma in terms of their general features and organization. This diagnosis is also described in different parts of the intestine of five cats

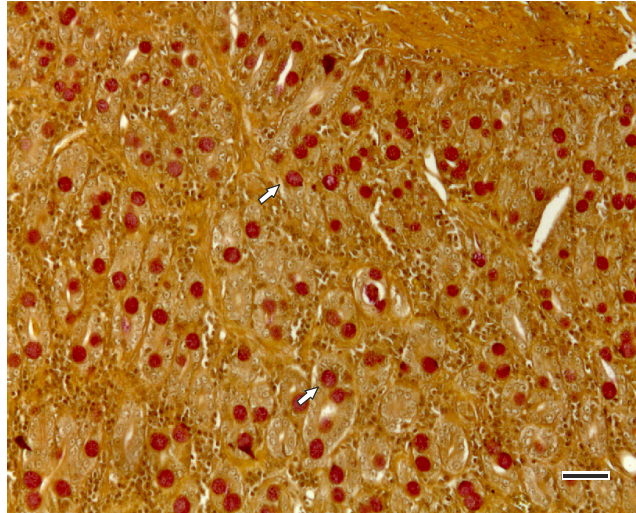


Fig. 6. Mucin positive cells (arrows). Bar= 40 μ m), Mayer's mucicarmine stain.

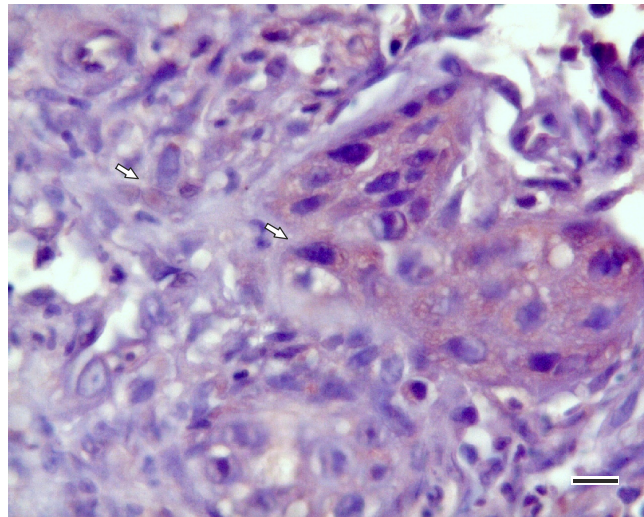


Fig. 7. Her2neu positivity (arrow) in anaplastic tubular cell's cytoplasm. Bar=40 μ m, ABC-P.

in a retrospective study (Cribb, 1988) but no information about invasive tumour cells and inflammatory cells was given in that report.

There are several reports about the immunohistochemical features of small intestinal adenocarcinoma in dogs and humans (Chanyapat *et al.*, 1999; Ramos-

Vara & Miller, 2002;Wendum *et al.*, 2003, Anita *et al.*, 2012). Positivities in the cytoplasm and the of anaplastic cells membranes are evaluated by using cy-tokeratin, vimentin, COX-2, hepatocyte paraffin-1 and epithelial membrane anti-gen. In the current case, a different ap-proach was used. The malignancy of tu-

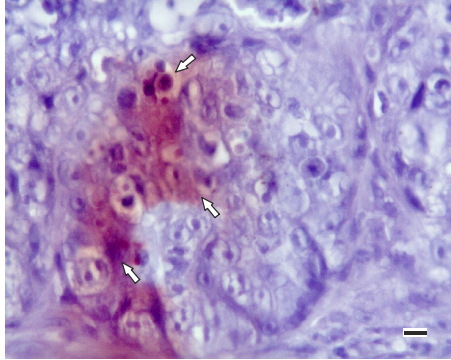


Fig. 8. CEA positivity (arrow) in anaplastic tubular cell's cytoplasm. Bar=30 μ m, ABC-P.

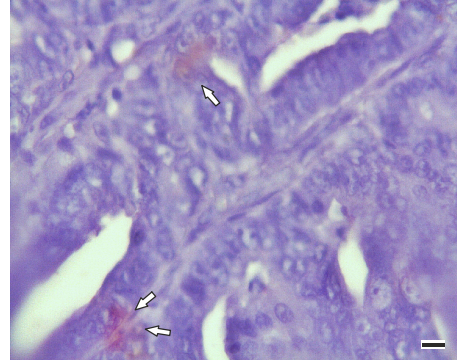


Fig. 9. Ki67 positivity (arrow) in anaplastic tubular cell's cytoplasm. Bar=35 μ m, ABC-P.

mour cells was used to show high proliferation as indicated by Damasceno *et al.* (2012). Almost all nuclei of anaplastic epithelial cells have reacted with Ki67 in many areas. The second useful marker was Her2neu, which is a kind of epidermal growth factor and definitive prognostic marker used to indicate invasive traits of mammary adenocarcinoma (Patrana *et al.*, 2012). It has been also used in colorectal adenocarcinoma in a dog for the same purpose and anaplastic epithelium membranes were found mildly positive (Damasceno *et al.*, 2012). In the present case, Her2neu was found in both membranes and cytoplasm of anaplastic glandular epithelial cells in almost all areas. Highly anaplastic cells were interpreted as predictors of poor prognosis. Additionally, CEA positivities in cytoplasm confirmed malignancy. In this context, similar results were obtained in the duodenum in colorectal patients by Fernandes & Matos (2002). It has been suggested that CEA could be another marker equally sensitive and reliable as others. The p63, which is similar to the p53 protein, did not react in any anaplastic cells in the current case. Also, p53 has not reacted in anaplastic

cells in the study of Tollenaar *et al.* (1998).

In conclusion, the characteristics of the tumour described in the current case are different from those of previous studies. The tubular adenocarcinoma of the duodenum is the first reported case despite that other intestinal tumours are commonly seen in Siamese cats. Its histological type and immunohistochemical features are different although in line with generally acknowledged traits reported in the literature. Therefore, this case will possibly provide meaningful contributions to pathologists.

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