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NOTE

Pathology

Diffuse leiomyomatosis with circumferential thickening of the gastrointestinal wall, resembling human diffuse leiomyomatosis, in a young miniature dachshund

Mizuki KURAMOCHI[1)], Takeshi IZAWA[1)]*, Mutsuki MORI[1)], Shunsuke SHIMAMURA[2)], Terumasa SHIMADA[2)], Mitsuru KUWAMURA[1)] and Jyoji YAMATE[1)]

[1)]Laboratory of Veterinary Pathology, Osaka Prefecture University, 1-58 Rinku-Ourai-Kita, Izumisano, Osaka 598-8531, Japan

[2)]Veterinary Medical Center, Osaka Prefecture University, 1-58 Rinku-Ourai-Kita, Izumisano, Osaka 598-8531, Japan

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ABSTRACT. Leiomyoma is the most common mesenchymal tumor in the gastrointestinal (GI) tract. Leiomyomas usually have a single or multinodular mass of various sizes, and affected animals can develop alimentary symptoms depending on the location and size. A 3-year old female miniature dachshund died after a history of refractory rectal prolapse, esophagectasis and aspiration pneumonia. At necropsy, the GI wall at the gastroesophageal and anorectal junctions was circumferentially thickened. Histologically, both GI lesions were composed of bundles of well-differentiated smooth muscles without mass formation or invasive growth. The neoplastic cells had little cellular atypia and low proliferative activity, and were positive for α-smooth muscle actin. The lesions were diagnosed as diffuse leiomyomatosis with circumferential thickening of the GI wall and has not been described in the veterinary literature.

KEY WORDS: anorectal junction, gastroesophageal junction, gastrointestinal tract, leiomyoma

Mesenchymal tumors of the gastrointestinal (GI) tract, originating from smooth muscle or nerve tissues, are common in animals [14]. Smooth muscle tumors are classified as benign leiomyoma and malignant leiomyosarcoma; leiomyoma is more common than leiomyosarcoma [1, 14]. Leiomyomas typically affect elder male dogs (age range: 8–17 years) [1, 14] and the gastric cardia and gastroesophageal junction are the most common sites [1, 14]. Depending on the tumor size and location, leiomyomas can cause clinical symptoms including vomiting, anorexia, tenesmus and obstruction, although they are often found incidentally at postmortem examination [1]. GI leiomyomas typically have a single to multiple solitary nodules located in the muscular layer. Microscopically, they are composed of bundles of well-differentiated smooth muscle cells without invasion into the adjacent tissue [1]. To our knowledge, there are few reports on leiomyoma without forming mass in veterinary medicine. Here we describe a canine case of multiple GI leiomyomas with circumferential thickening of the wall at the gastroesophageal and anorectal junctions in a young dog.

A 3-year-old female miniature dachshund was presented with a refractory rectal prolapse. Dyschezia was presented due to rectal prolapse. Laboratory findings indicated proteinuria (the urine protein/creatinine ratio [UPC]: 6.64). Other hematology and biochemistry parameters were within their respective reference intervals. The prolapsed rectum was surgically repositioned, however it recurred after surgery. Kidney biopsy was performed at the same time and membranous glomeruronephlopathy was suspected by a contract diagnostic laboratory. After that, stool extraction was performed periodically for the constipation. UPC decreased to 1.98 after an administration of mycophenolate mofetil (mycophenolate mofetil capsules, Pfizer, Tokyo, Japan). Chronic regurgitation and vomiting were present 5 months after initial presentation. Computed tomography revealed a megaesophagus and a thickening of the wall of the gastroesophageal junction. The gastrostomy tube was applied, because of the passage disturbance from the esophagus to the stomach. However, the dog died with aspiration pneumonia 8 months after the initial presentation and a necropsy was performed.

Grossly, the GI wall at the gastroesophageal and anorectal junctions was circumferentially thickened. The thickened wall at the

*Correspondence to: Izawa, T.: izawa@vet.osakafu-u.ac.jp

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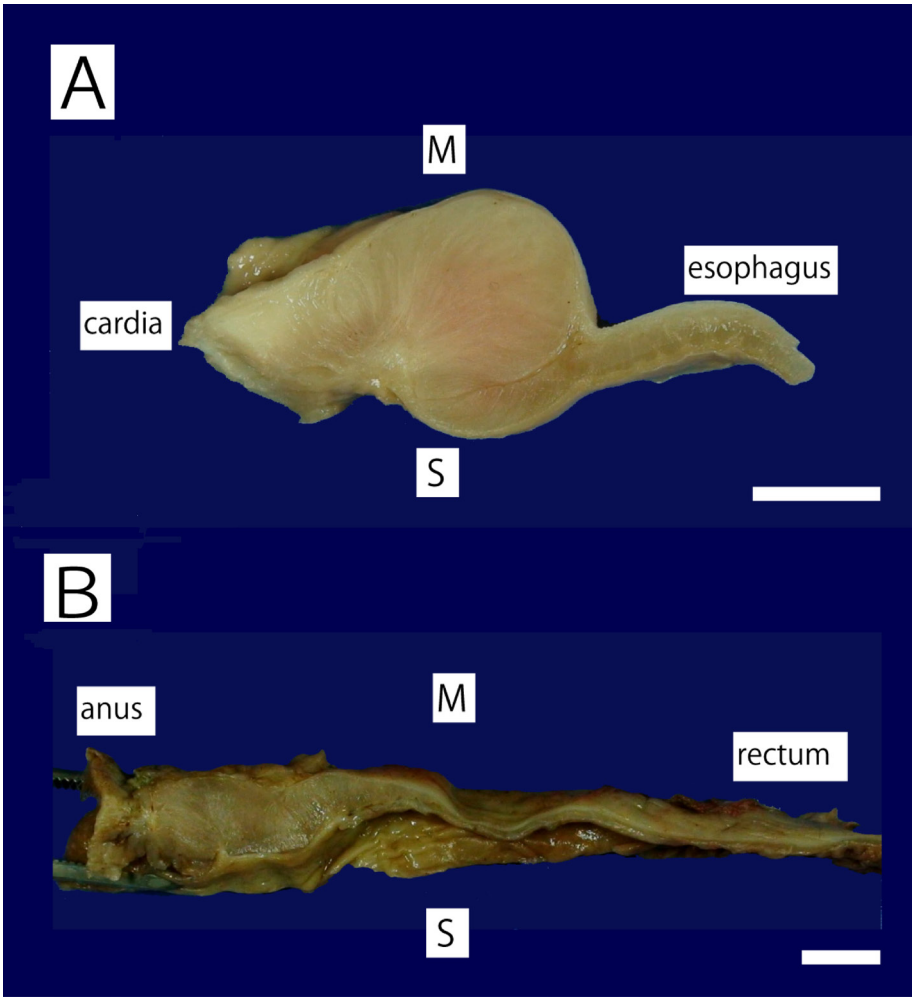

Fig. 1. Macroscopic images of the gastrointestinal wall. The longitudinal cut surface of (A) gastroesophageal and (B) the anorectal junctions show diffuse thickening of the smooth muscle layer. M; mucosa, S; serosa. Bar=1 cm.

gastroesophageal junction was 3 cm in length and 2 cm in thickness (Fig. 1A), while that at the anorectal junction was 2 cm in length and 1 cm in thickness (Fig. 1B); stenosis of the lumen was observed at both sites. A hard, whitish nodular lesion of 3 cm in diameter was seen in the right anterior lobe of the lung, which was diagnosed histopathologically as aspiration pneumonia. There were no gross abnormalities in other organs including the liver, spleen, kidneys, heart, trachea and adrenal glands. Tissues were fixed in 10% neutral-buffered formalin, routinely processed and embedded in paraffin. Histological sections cut at 5 μm were stained with hematoxylin and eosin (HE). The kidney sections were subjected to periodic acid methenamine (PAM) silver stain. Immunohistochemical analyses were performed using monoclonal antibodies against α-smooth muscle actin (α-SMA; clone 1A4, 1:1,000, Dako, Glostrup, Denmark), dog immunoglobulin (dog Ig; 1:200) and Ki-67 (clone MIB-1, 1:500, Dako). After dewax, sections for Ki-67 were pretreated by microwave for 20 min in 0.01 M citrate buffer (pH 6.0) as previously reported [4]. Sections were incubated with 3% H_2O_2 in phosphate-buffered saline (PBS) for 10 min to quench endogenous peroxidase. Thereafter, the sections were treated with 5% skimmed milk in PBS for 30 min and incubated with each primary antibody for 1 hr at room temperature, followed by an incubation with peroxidase-conjugated secondary antibody (Histofine Simple Stain MAX PO; Nichirei, Tokyo, Japan). Positive reactions were detected with 3, 3′-diaminobenzidine (DAB Substrate Kit; Nichirei). Sections were counterstained lightly with hematoxylin.

Microscopically, both of the GI lesions were located in the muscular layer without invasion into the serosa or submucosa (Fig. 2A, 2D). The inner muscular layer was thickened at both sites, while the outer muscular layer was partly atrophied with mild inflammatory infiltrates. In the esophagus adjacent to the lesion, the striated muscle of the outer layer was also compressed and atrophied by the thickened smooth muscle. The thickened lesions were composed of bundles of elongated cells with oval nuclei, abundant eosinophilic cytoplasm and indistinct cell border, showing morphological features of well-differentiated smooth muscle cells (Fig. 2B, 2E). They were positive for α-SMA (Fig. 2C, 2F). The smooth muscle cells in the thickened lesion had low mitotic count (1 in 10 high power fields) and low Ki-67 count (2 in 10 high power fields). Extensive suppurative pneumonia

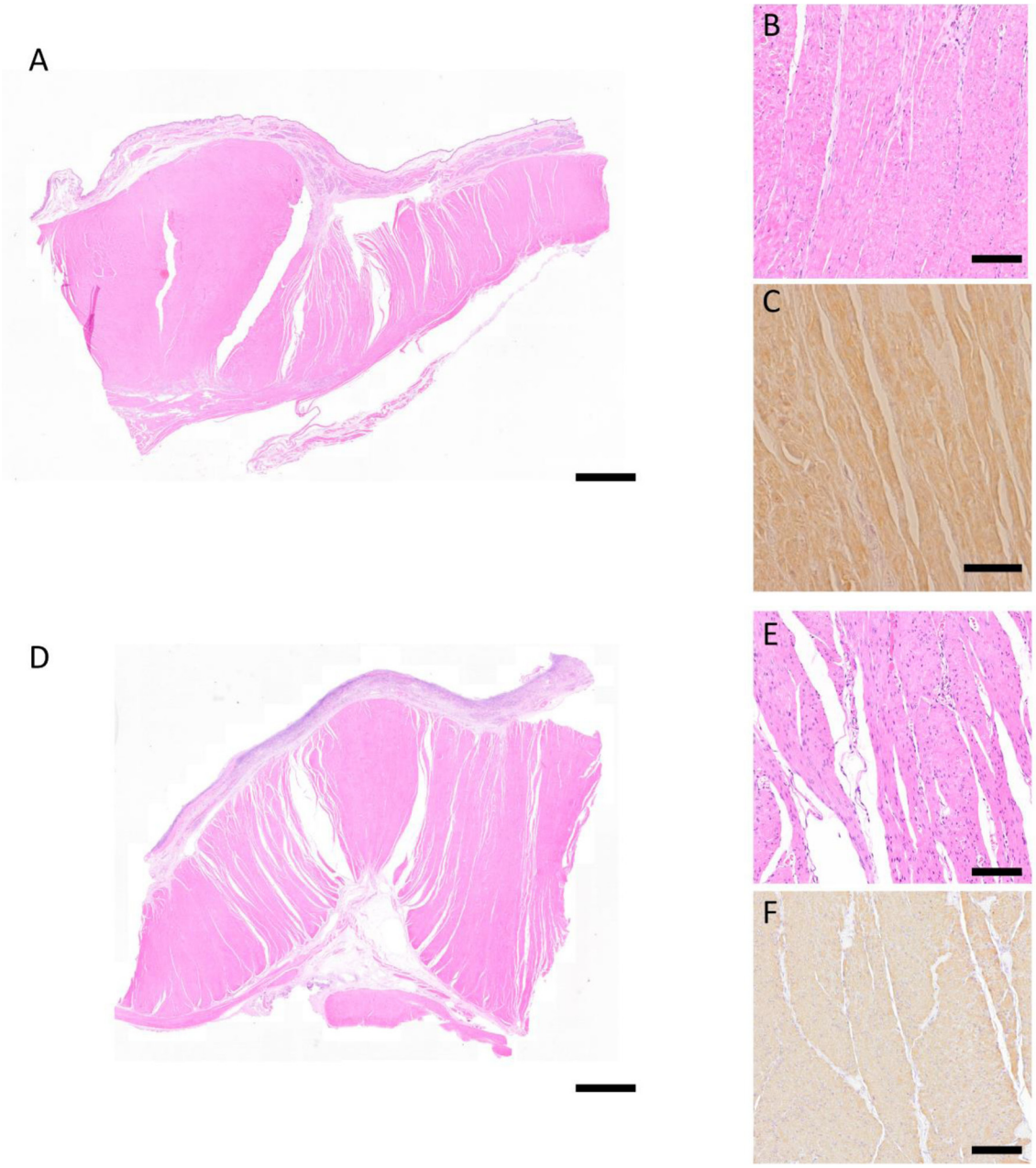

Fig. 2. Microscopic images of the gastrointestinal lesions. The thickened lesions at (A) the gastroesophageal and (D) the anorectal junctions are composed with well-differentiated neoplastic smooth muscle cells without invasion into the adjacent serosa and submucosa. HE stain. Bar=2 mm. (B) Higher magnification of A. HE stain. Bar=100 μm. (C) The neoplastic cells are positive for α-smooth muscle actin. Bar=50 μm. (E) Higher magnification of D. HE stain. Bar=100 μm. (F) The neoplastic cells are positive for α-smooth muscle actin. Bar=100 μm.

with food debris and bacterial colonies in the bronchial lumen was observed in the right lung. Histopathologically, there were several glomeruli with hyalinization (sclerosis) (Supplementary Fig. 1A). Thickening of glomerular basement membrane is rarely shown with PAM stain (Supplementary Fig. 1B), and immunoglobulin deposition was not detected (Supplementary Fig. 1C) in the glomerulus. Additionally, activation of mesangial cells with α-SMA expression [6] was not observed (Supplementary Fig. 1D).

Based on the gross and histological findings, the present case was diagnosed as diffuse leiomyomatosis, characterized by circumferential thickening of the GI wall at the gastroesophageal and anorectal junctions in a young dog. The thickening of gastroesophageal wall and anorectal wall are likely to be responsible for aspiration pneumonia due to esophageal dilation and refractory rectal prolapse, respectively.

The present case shares pathological similarities with diffuse leiomyomatosis in humans. Human leiomyomatoses on the alimentary tract have been reported sporadically [5, 11] or as lesions associated with Alport syndrome [2, 3, 13]. Affected site of human sporadic diffuse leiomyoma is mainly esophagus [11], showing vomiting, megaesophagus and achalasia as clinical signs.

Histopathologically, both sporadic and Alport syndrome-associated diffuse leiomyomatoses are characterized by circumferential proliferation of well-differentiated smooth muscle cells with low mitotic activity [10], which is the key diagnostic feature to differentiate diffuse leiomyomatoses from leiomyomas that usually form nodular mass. Diffuse leiomyomatosis is a minor phenotype in Alport syndrome most commonly involving the esophagus; the rectum, respiratory and female genital systems may also be affected [3, 10]. In patients with Alport syndrome, clinical findings are mainly associated with renal disorder and auditory disturbance due to abnormality in collagen generation [10, 13]. Irregular thickening of the lamina densa of the glomerular basement membrane, confirmed with electron microscope, is an important diagnostic criterion [10, 15]. Clinically, most (81%) of patients with Alport syndrome have hematuria with or without proteinuria [7] and 11% patients have proteinuria alone; the proteinuria was also seen in the present canine case. However, the glomerular lesions were focal and modest in the present case without relevance to renal lesions in human Alport syndrome [14, 15]. Patients with sporadic esophageal leiomyomatosis, without personal or familial history of Alport syndrome, are reported not to have renal disorders [5, 11].

In veterinary medicine, GI disorders with circumferential thickening of the smooth muscle wall have been rarely reported in horses [8, 9] and a dog [12]. In a retrospective study on horse cases, Friesian horses of varying ages had a higher prevalence of circumferential thickening of smooth muscle layer of the caudal esophagus, often associated with megaesophagus; such esophageal lesions were rare in other horse breeds [8]. Renal lesions were not evident in the affected Friesian horses. Another study reported a circumferential thickening of smooth muscle layer of the duodenum in 2 horses that were histopathologically diagnosed as leiomyosarcoma [9]. In a canine case, a 10-year-old female Rottweiler had a circumferential thickening of smooth muscle at the gastroesophageal junction with esophageal distension; renal abnormality was not described in this case [12].

The age of the present case (3 years) is quite younger than the median age of GI leiomyomas in dogs (16.8 years) in the literature [14], raising a possibility that the present case might be related with some genetic background. Unfortunately, genetic analysis could not be performed in the present case and the pedigree could not be chased. Further accumulation of case studies would contribute to understanding this unique GI disorder in the dog.

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