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Original article

Clinical relationship between histopathological necrotic/partial necrotic findings and disease condition of gallbladder mucoceles in dogs

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Abstract

Gallbladder mucocele (GM) is a common extrahepatic biliary disease recognized in dogs and is defined as the expansion and extension of the gallbladder by an accumulation of semi-solid bile or bile acid. Histopathological diagnosis of necrotizing cholecystitis and transmural coagulative necrosis of the gallbladder wall shows poor prognosis. Conversely, histopathological diagnosis with partial necrotic findings is often achieved. We hypothesized that histopathological partial necrosis of the gallbladder wall is the primary lesion of necrotic cholecystitis or transmural ischemic necrosis. Therefore, we investigated the relationship between histopathological necrosis/partial necrosis findings and their clinical conditions. We retrospectively analyzed 55 dogs diagnosed with GM that had undergone cholecystectomy at the Yamaguchi University Animal Medical Center. The group with histopathological necrosis/partial necrosis of the gallbladder wall showed elevated levels of preoperative white blood cells, alanine transaminase, alkaline phosphatase, γ-glutamyltransferase, total bilirubin, and C-reactive protein compared to the non-necrotic group. Partial necrosis of the gallbladder wall may affect the progression of the disease and hematological abnormalities. Additionally, all death cases until 2 weeks were included in the histopathological necrosis/partial necrosis group. In this study, we found that poor prognosis factors were associated with partial necrosis of the gallbladder wall. Furthermore, these cases of partial necrosis showed elevated levels of blood test parameters. These results suggest that necrosis of the gallbladder wall is associated with poor prognosis and poor pathophysiological conditions.

Key words: canine, cholecystectomy, gallbladder mucoceles, necrosis, gangrenous cholecystitis

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Introduction

Gallbladder mucocele (GM) is a common extrahepatic biliary disease recognized in dogs and is defined as the expansion and extension of the gallbladder by an accumulation of semi-solid bile or bile acid (Besso et al. 2000, Mesich et al. 2009). Decreasing motility of the gallbladder, inflammation of the gallbladder wall, hyperlipidemia and abnormality of gallbladder mucous have been suggested as factors that lead to the development of GMs (Kovatch et al. 1965, Tsukagoshi et al. 2012, Xenoulis 2014). GMs appear in middle and advanced aged Shetland sheepdogs, Cocker spaniels, and Miniature Schnauzers; these breeds are predisposed to GM (Besso et al. 2000, Aguirre et al. 2007, Norwich 2011). Patients with GM show various symptoms, such as vomiting and anorexia. However, many of them are asymptomatic, and when symptoms develop, they are often extrahepatic biliary obstruction, rupture of the gallbladder, and subsequent peritonitis (Newell et al. 1995, Worley et al. 2004). The clinical diagnosis of GM is made based on symptoms, blood examinations, and ultrasound (Borusewicz et al. 2016). Histopathological diagnosis of GM is associated with an abnormality of mucus-secreting cells in the mucosa of the gallbladder wall (Kovatch et al. 1965). Mucosal hyperplasia of the gallbladder wall is also often seen in the histopathological diagnosis of GM (Smalle et al. 2015).

In veterinary medicine, GM patients with histopathological necrotic findings are common, and histopathological diagnosis of necrotic cholecystitis and transmural ischemic necrosis is supposed to have a poor prognosis (Center 2009). Interestingly, histopathological findings of gallbladder wall necrosis are rare in humans. One major pathophysiology of gallbladder wall necrosis is gangrenous cholecystitis (GC), which is a serious complication of acute cholecystitis (AC) (Bingener et al. 2005). In humans, many studies have reported the comparison of clinical conditions between AC and GC (Bennett et al. 2002, Contini et al. 2004).

Some reports in veterinary medicine have suggested pancreatitis, high serum lactate concentration, and high white blood cell (WBC) count as poor prognostic factors post-cholecystectomy (Amsellem et al. 2006, Uno et al. 2009, Malek et al. 2013). However, no reports have compared its histological types and clinical findings.

We hypothesized that histopathological partial necrosis of the gallbladder wall is the primary lesion of necrotic cholecystitis or transmural ischemic necrosis. Our purpose of this study is to investigate the correlation between disease conditions and histological necrosis/partial necrosis.

Materials and Methods

Patients and diagnosis

As previously reported, GM is common in old and small breed dogs. The breed predisposition for GM included Shetland sheepdog, Cocker Spaniels, Scottish Terries, and Miniature Schnauzers. In this study, we retrospectively analyzed the medical record data on blood test findings and histopathological diagnosis of cases of GM at the Yamaguchi University Animal Medical Center between January 2008 and September 2017 that underwent cholecystectomy. The mean age at the time of surgery was 9.3 (1.3-17.5) years. According to medical record, total of 55 dogs (30 males and 25 females) were included in this study. The breeds were Chihauha (9), Shiba Inu (7), Miniature Dachshund (5), Pomeranian (5), Miniature Schnauzer (4), Shetland Sheepdog (3), Maltese (2), Papillon (2), Toy Poodle(2), Shih Tzu (2), Mongrel dog (2), Cavalier King Charles Spaniel (2), American Cocker Spaniel (2), Yorkshire Terrier (1), Boston Terrier (1), Beagle (1), Pug (1), Dalmatian (1), Scottish Terrier (1), West Highland White Terrier (1) and Bernese Mountain Dog (1). We analyzed all the dogs diagnosed with GM by clinical symptoms, blood tests, and ultrasonography. All the dogs were diagnosed with GM via ultrasonographic examination when echogenic bile did not reposition itself with the change in the dog's position to confirm the absence of gravity-dependent bile movement, as described in a previous study (Choi et al. 2014). Ultrasonographic examination was performed by a clinical veterinarian using an ultrasound machine (EUB-8500, or HI VISION Preirus, HITACHI, Tokyo, Japan) equipped with a microconvex transducer.

Preoperative examination

Patients underwent preoperative blood examination for parameters, namely WBC, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), γ-glutamyletransferase (GGT), total bilirubin (T-Bil), total cholesterol (T-Chol), lipase (Lip), glucose (Glu), electrolyte (Na, K, Cl), and C-reactive protein (CRP). All complete blood cell and biochemistry examinations were performed using the same equipment (XT-2000i, SYSMEX, Kobe, Japan) (DRI-CHEM 7000V, FUJIFILM, Tokyo, Japan).

Histopathological analysis

All patients underwent cholecystectomy. To compare each clinicopathological finding according to histopathological classification, all resected gallbladders underwent a histopathological examination at a private company (IDEXX Laboratories, Tokyo, Japan) or the

laboratory of pathology at the Yamaguchi University. In addition, to compare clinical conditions between necrosis/partial necrosis patients and non-necrosis patients, necrotic cholecystitis or transmural ischemic necrosis as well as any partial necrosis findings in histopathological examinations were grouped as necrotic/ /partial necrotic group (NG). In contrast, histopathological diagnosis and comments with no necrosis findings were grouped in the non-necrotic group (NNG).

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, The United States). Patient age was expressed as a median (range). The results of blood examinations were expressed in terms of average ± standard error. The two groups were compared using the *F*-test to determine homoscedasticity in each group. For equal variance, we performed an independent *t*-test, whereas for unequal variance, we performed Welch's test to compare the two groups. Data were considered statistically significant when the p-value was ≤ 0.05.

Results

Perioperative mortality rate

Gallbladders of all patients were resected and histopathological examination was performed. The appearance of the gallbladder varied, but typically showed extended gallbladder and jelly-like conten (Fig. 1a). Four patients died within 2 weeks after the operation, and the postoperative mortality rate was 7.2% (4/55).

Histopathological diagnosis

Resected gallbladders were cut in the longitudinal direction and histopathological analysis was performed (Fig. 1b-e). The histopathological evaluation of necrosis was classified as "coagulation necrosis" if the pattern of necrosis was ischemic in any layer, "transmural ischemic necrosis" if all layers of the gallbladder wall were necrotic, and "necrosis of the gallbladder" such as only mucosa were necrotic. Necrotic cholecystitis was also diagnosed when there was inflammation with severe gallbladder wall necrosis. Purulent cholecystitis was diagnosed when there was clear histopathological infection. As a result, histopathological diagnoses of gallbladders included mucosal hyperplasia of the gallbladder wall (23), lymphoplasmacytic cholecystitis (9), necrosis of the gallbladder (6), normal gallbladder (3), coagulative necrosis of the gallbladder (3), lymphocytic cholecystitis (2), necrotic cholecystitis (2), transmural ischemic necrosis (1), purulent cholecystitis (1), and others (5).

Comparison of the signalment characteristics of dogs between histopathological non-necrosis and necrosis/partial necrosis groups

The patients were categorized as NNG and NG based on histological necrosis findings. As a result, 39 and 16 cases were categorized as NNG and NG, respectively. Histopathological diagnoses of gallbladder necrosis in NG cases included necrosis of the gallbladder (6), mucosal hyperplasia of the gallbladder wall (4), coagulative necrosis of the gallbladder (3), necrotic cholecystitis (2), and transmural ischemic necrosis (1). All four death cases were classified as NG (Table 1). Other cases classified as NG or NNG are shown in Table 2.

Comparison of blood examination in dogs between NNG and NG

Preoperative blood examination showed elevated levels of WBC (13/55), AST (20/51), ALT (40/55), ALP (45/55), GGT (36/53), T-Bil (14/47), T-Chol (18/45), Lip (9/42), and CRP (15/55) levels. Patients in the NG had a significantly greater elevation of WBC, ALT, ALP, GGT, T-Bil, and CRP levels than patients in the NNG. In contrast, patients in the NNG had significantly greater elevations of chloride concentration than those in the NG (Table 2).

Discussion

As previously reported, GM is common in old and small breed dogs. The breed predisposition for GM included Shetland sheepdog, Cocker Spaniels, Scottish Terries, and Miniature Schnauzers (Mealey et al. 2010). However, Shiba-Inu was overrepresented in this study. Therefore, Shiba-Inu may be a breed predisposed to GM. However, this has not been previously reported, suggesting that Shiba-Inu is a native species of Japan.

Reportedly, the mortality rate of GM patients who required post-surgical treatment was 21.7%-40% (Pike et al. 2004, Smalle et al. 2015). In two recent studies (Worley et al. 2004, Malek et al. 2013), the immediate postoperative mortality rates were reported as 7% and 32%, respectively. In this study, the total mortality rate was 7.2% (4 cases) at 2 weeks. A previous study revealed that GM patients that underwent surgical treatment and survived for 14 days showed a better prognosis (Smalle et al. 2015). Additionally, all four cases were included in NG. In this study, we also observed poor prognostic factors for histopathological diagnosis

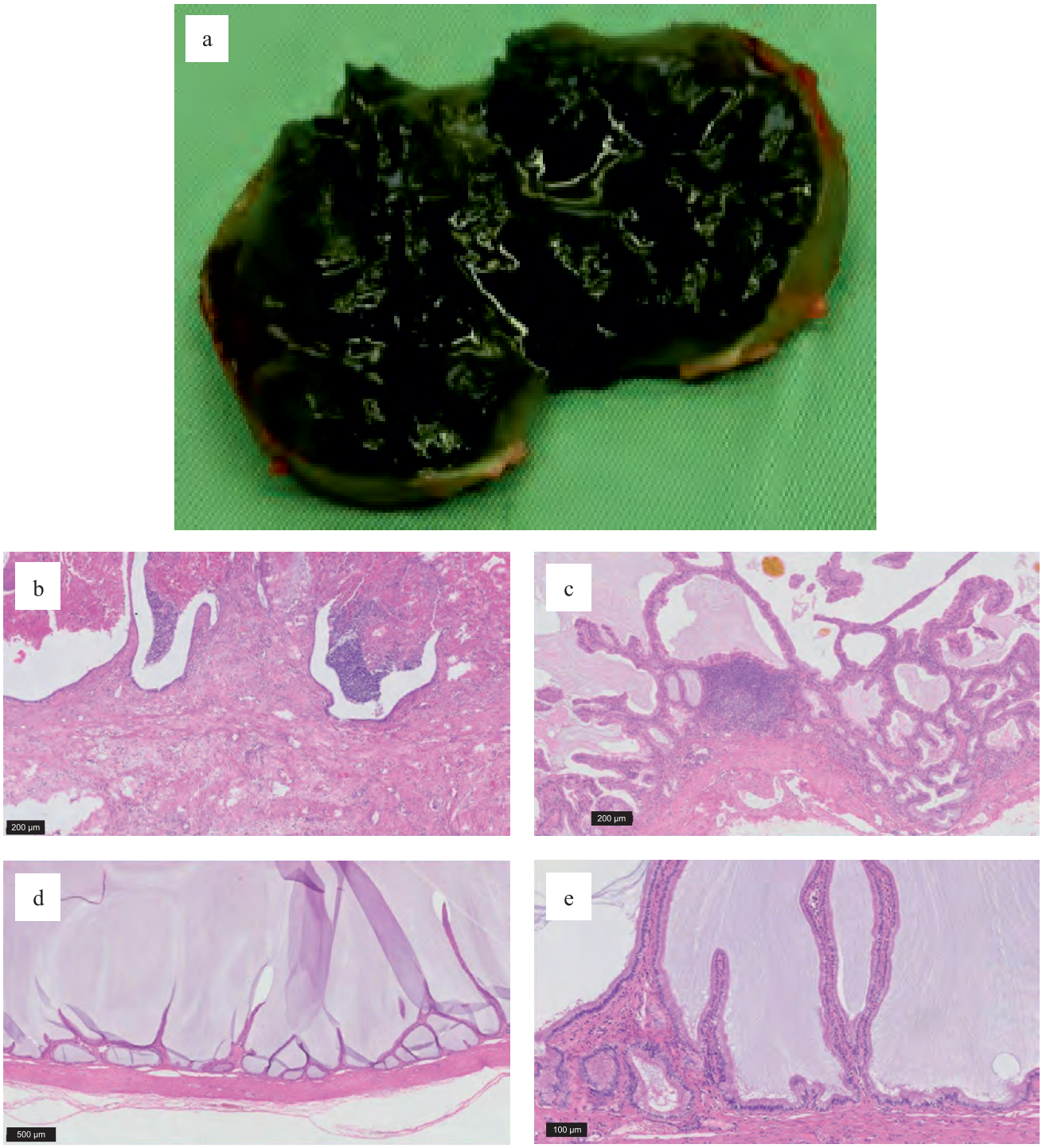

Fig. 1. Appearance and histopathology of canine gallbladder mucoceles. The distended gallbladder with jelly-like contents (a). The gallbladder mucosa is widely necrosed and replaced by red blood cells and degenerated cells. This case was diagnosed as necrosis of the gallbladder (b) (Scale bar: 200 µm). The gallbladder mucosa was hyperplastic, but inflammatory cells, mainly lymphocytes and plasma cells, were observed in the lamina propria. This case was diagnosed as lymphoplasmacytic cholecystitis (c) (Scale bar: 200 µm). Overall hyperplasia of the gallbladder mucosa, including cystic structures, was recognized. This case was diagnosed as mucosal hyperplasia of the gallbladder wall (d) (Scale bar: 500 µm). Overall hyperplasia of the gallbladder mucosa was recognized. Some lymphocytes and plasma cells were observed in the lamina propria. This case was also diagnosed as mucosal hyperplasia of the gallbladder wall (e) (Scale bar = 100 µm).

of necrotic cholecystitis and coagulative necrosis of the gallbladder, in accordance with a previous report (Center 2009).

In human studies, histopathological necrosis of the gallbladder wall is recognized as GC. The pathophysiology of GC is understood as primary acute biliary

Table 1. Comparison of clinical conditions and histological diagnosis between non-necrotic and necrotic/partial necrotic groups of dogs.

		Non-Necrotic group (39)	Necrotic/Partial necrotic group (16)
Age in years - median (range)		9.4 (1.3 to 17.5)	9.1 (3.6 to 13.7)
Sex	male	19	11
	female	20	5
Bleed (N)		Chihauha (7)	Shiba Inu (3)
		Shiba Inu (4)	Pomeranian (3)
		Miniature Dachshund (5)	Chihauha (2)
		Pomeranian (2)	Maltese Dog (2)
		Papillon Dog (2)	Mixed Bleed (2)
		Shetland Sheep Dog (2)	Others (4)
		Shih Tzu (2)	
		Miniature Schnauzer (2)	
		Others (13)	
Number of death until 2 weeks		0	4
Histopathological diagnosis (N)		Mucosal hyperplasia of gallbladder wall (19)	Necrosis of gallbladder (6)
		Lymphoplasmacytic Cholecystitis (9)	Mucosal hyperplasia of gallbladder wall (4)
		Lymphocytic Cholecystitis (2)	Coagulatic necrosis of gallbladder (3)
		Others (5)	Necrotic cholecystitis (2)
		Normal gallbladder (3)	Transmural ischemic necrosis (1)
		Purulent cholecystitis (1)	

Table. 2 Comparison of blood examination between NNG or NG

Unit	All patients	n	NNG	n	NG	n	p-value
WBC (μl)	14410±10748	55	11552±8859	39	21376±11720	16	0.0016*
AST (IU/l)	103±194	51	55±109	35	208±279	16	0.0571
ALT (IU/l)	337±545	55	162±178	39	805±813	16	0.0081*
ALP (IU/l)	3506±5762	55	1254±1472	39	8993±7889	16	0.0081*
GGT (IU/l)	76±128	53	50±134	37	135±86	16	0.0103*
TBIL (mg/dl)	1.1±2.2	47	0.5±0.8	31	2.4±3.2	16	0.0038*
CHOL (mg/dl)	298±114	45	285±114	32	329±109	13	0.2523
GLU (mg/dl)	102±17	53	100±13	38	107±23	15	0.1469
Na (mEq/l)	148±2	55	148±2	39	148±3	16	0.4677
K (mEq/l)	4.0±0.5	55	4.1±0.5	39	3.8±0.6	16	0.094
Cl (mEq/l)	110±7	55	112±6	39	104±6	16	< 0.0001*
LIP (IU/l)	178±292	42	201±344	29	125±88	13	0.2829
CRP (mg/dl)	1.9±3.7	55	0.9±2.4	39	4.3±5.1	16	0.002*

* $p \leq 0.05$

tract obstruction and dominant blood vessel ischemia caused by gallbladder enlargement. Gallbladder wall tension with secondary phospholipase release with epithelial injury due to increased intra-cholecystic pressure (Fry et al. 1981), and secondary ischemic necrosis of the wall caused by cystic wall thrombosis or vascular compromise secondary to sustained obstruction of the cystic duct (Nikfarjam et al. 2011), have also been reported. GC is a known complication of AC and acute biliary tract obstruction by cholelithiasis is the most common mechanism of AC (Friedman 1993). GC patients show a higher mortality rate than normal AC patients (Morfin et al. 1968). (Fagan et al. 2003) reported age ≥51 years, African-American race, history of diabetes mellitus, WBC > 15,000, AST > 43 μ/L, ALT > 50 μ/L, ALP > 200 μ/L, Lip > 200 μ/L, and pericholecystic fluid on ultrasonography as prognostic factors for GC. Another report showed that high CRP level is a risk factor for GC (Chaudhry et al. 2011). Conversely, there are few studies on the mechanism of onset and histopathological characterization of GM in dogs. Previous reports showed that histopathological diagnosis of necrotic cholecystitis and transmural ischemic necrosis are considered to be poor prognostic factors for GM patients (Center 2009). Additionally, some laboratory findings, such as elevated levels of WBC and GGT, are correlated with postoperative mortality rate (Amsellem et al. 2006, Aguirre et al. 2007). In addition, it is considered that dogs with gallbladder mucoceles and hypothyroidism treated medically and have good prognosis (Walter et al. 2008). According to these reports, the recognition of the clinical severity of GM before surgery and the determination of the necessity and timing of surgical intervention remain unknown.

In this study, abnormalities in blood examinations were observed in many cases. Additionally, NG patients showed significant elevation of preoperative WBC, ALT, ALP, GGT, T-Bil, and CRP levels compared with NNG patients. This result may suggest that necrosis of the bladder wall negatively affects GM. However, patient age and AST and Lip levels were not high/ /elevated in NG patients. Human GC studies have revealed the details of the mechanisms underlying the development of GC from AC. Unlike those in human medicine, certain mechanisms of GM development have not been completely revealed in veterinary medicine. Hence, the mechanism of the development of GM may be different from that of GC in humans. On the other hand, the fact that there is a connection between the necrosis of the gallbladder wall and poor prognosis or clinical condition seems to be correlated by previous human and veterinary studies and our contribution. In addition, although the exact prognosis is unknown, it has been reported that thrombosis of the gallbladder artery causes necrosis in canine gallbladder mucoceles (Holt et al. 2004). Further studies on the mechanism of necrosis of the gallbladder wall may improve treatment outcomes of GM.

Conclusion

Our results suggest that necrosis of the gallbladder wall is associated with poor prognosis and poor pathophysiological conditions.

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