Background/Aims: Previous studies have reported that endotoxemia is associated with pathogenesis and complications in cirrhosis. Endotoxin stimulates the secretion of inflammatory cytokines, which contributes to the development of complications. In addition, endotoxin easily invades the gut barrier system because of the increased intestinal permeability due to portal hypertensive enteropathy. In this report, we explored changes in cytokine levels and intestinal permeability and measured the thickness and elasticity of the bowel wall using ultrasonography in cirrhotic patients.

Methods: We enrolled 40 patients with cirrhosis classified as Child-Pugh B or C and 20 healthy volunteers. Abdominal ultrasonography examinations were used to evaluate bowel wall parameters in the ascending colon and terminal ileum. Intestinal permeability was measured using dual sugar absorption tests with lactulose and mannitol. Levels of tumor necrosis factor (TNF)-α and IL-10 were determined from blood samples. We compared these outcomes between cirrhotic patients and healthy controls and between Child-Pugh B and C patients. In addition, we explored the correlation between cytokine levels, intestinal permeability ratio, and bowel wall parameters in cirrhotic patients.

Results: In cirrhotic patients, the ascending colon wall elasticity decreased (20.4 vs. 10.9 kPa, \( p = 0.048 \)) and the terminal ileum wall thickness increased (4.2 vs. 1.9 mm, \( p < 0.001 \)). The intestinal permeability ratio and levels of the cytokines TNF-α and IL-10 increased (0.219 vs. 0.017, \( p < 0.001 \); 22.47 vs. 13.48 pg/mL, \( p < 0.001 \); and 14.91 vs. 8.57 pg/mL, \( p = 0.019 \), respectively) in cirrhotic patients. However, there were no significant differences between Child-Pugh classes and no significant correlations between bowel wall parameters and intestinal permeability or cytokine levels.

Conclusions: Ultrasonography revealed bowel wall thickening and decreases in elasticity; in addition, intestinal permeability and cytokine levels increased in cirrhotic patients compared with healthy controls. (Korean J Med 2019;94:511-518)

Keywords: Liver cirrhosis; Permeability; Intestines; Ultrasonography
INTRODUCTION

Liver cirrhosis presents as various disease activities and causes numerous complications. Some patients suffer from severe complications, whereas others endure a stable clinical course over time. Cirrhosis complications are caused by increased hepatic resistance, splanchnic vasodilation, and the formation of varices, as well as other portosystemic collaterals. Recently, several studies have reported that bacterial translocation and endotoxemia are associated with the pathogenesis of cirrhosis complications [1-3]. Bacterial translocation from the intestine can cause serious infectious complications such as spontaneous bacterial peritonitis and pneumonia. Endotoxin, which is found in the cell walls of Gram-negative bacteria, stimulates the secretion of inflammatory and anti-inflammatory cytokines, as well as acute phase reactants such as tumor necrosis factor (TNF) and C-reactive protein [4]. These cytokines contribute to the progression of liver fibrosis and development of cirrhosis complications. Lin et al. [5] reported that endotoxin contributes to compromised immunity in cirrhotic patients through the expression of interleukin (IL)-10 and human leukocyte antigen-DR. Jain et al. [4] also reported elevated endotoxin and TNF levels in patients with hepatic encephalopathy with cirrhosis. These cytokines additionally induce increases in nitric oxide, leading to vasodilation. The hyperdynamic circulatory state due to vasodilation causes other serious complications such as ascites, variceal bleeding, hepatorenal syndrome, and hepatic encephalopathy. Bacteria and endotoxin easily invade the gut barrier in patients with cirrhosis because of increased intestinal permeability due to portal hypertensive enteropathy. Endotoxemia can increase intestinal permeability, which triggers a vicious cycle [6].

Bowel wall thickening is a common finding in abdominal computed tomography (CT) scans of patients with cirrhosis [7]. Gastrointestinal wall thickening can appear in many conditions such as ischemia, inflammation, hemorrhage, or neoplasm; however, in patients with cirrhosis, bowel wall thickening is caused by intestinal edema [8]. Portal hypertension plays an important role in intestinal edema, which in turn causes mucosal disintegration and increases intestinal permeability [9].

Ultrasonography (US) has several advantages compared to CT, such as wider availability, avoidance of radiation exposure, and the ability to assess the elasticity of the intestine. Several studies have explored bowel elasticity using US elastography in patients with Crohn’s disease [10-12]. These studies showed that bowel elasticity changes according to disease activity. We hypothesized that bowel wall edema and increased permeability would result in changes in bowel elasticity in patients with cirrhosis. To the best of our knowledge, bowel wall changes have not been assessed using US in patients with cirrhosis. Therefore, we assessed the relationship between intestinal permeability, inflammatory cytokines, and bowel wall changes using US in patients with cirrhosis.

MATERIALS AND METHODS

We prospectively enrolled 40 decompensated cirrhotic patients (Child-Pugh classifications B and C with 20 patients in each category) who were admitted to the gastroenterology ward or attending the outpatient clinic of the Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, and recruited 20 healthy volunteers from the health check-up center at Kangbuk Samsung Hospital. Cirrhotic patients were enrolled irrespective of their etiology. Healthy controls without chronic hepatitis B or C were enrolled.

Exclusion criteria included binge drinking, receiving antibiotics within 2 weeks, receiving drugs for constipation or synbiotics, diagnosis of malignancy, history of abdominal surgery, inflammatory bowel disease, co-morbidity with severe disease, and hypersensitivity to lactulose and mannitol. The diagnosis of cirrhosis was based on patient history, clinical findings, and endoscopic, laboratory, and imaging criteria. The severity of liver disease was assessed using the Child-Pugh classification and model for end-stage liver disease (MELD) score.

All participants provided informed consent for this study. The study protocol conforms to the ethical guidelines of the World Medical Association Declaration of Helsinki and was approved by Institutional Review Board of Kangbuk Samsung Hospital.

All participants’ blood was obtained on the day of enrollment in this study. Levels of TNF-α and IL-10 were assessed using
an immunology multiplex assay (Human Cytokine/Chemokine Magnetic Bead Panel; MilliporeSigma, Burlington, MA, USA) and an immunoassay analyzer (Luminex 200xPONENT System; MilliporeSigma) in accordance with the manufacturer’s instructions.

Intestinal permeability was assessed by measuring the level of lactulose and mannitol in the urine. The first urine sample was collected in the morning after overnight fasting (at least 8 hours) and after patients had drunk 5 g of lactulose and 1 g of mannitol with water. Patients then collected urine over 6 hours while drinking a cup of water hourly. The lactulose/mannitol ratio in the collected urine was assessed using an enzymatic assay with an intestinal permeability urine test kit (Genova Diagnostics, Inc., Asheville, NC, USA).

The thickness and elasticity of the bowel wall were measured at the anterior bowel wall of the ascending colon and terminal ileum by one radiologist (blinded) with 11 years of experience in abdominal US imaging using a 50-mm linear probe with a frequency range of 4–15 MHz with an Aixplorer ultrasound system (SuperSonic Imagine, Aix en Provence, France). The elasticity of the liver was also measured by the same radiologist using a convex transducer (C5–1 MHz) with a shear wave elastography instrument (Elastography point quantification; ElastPQ; Philips Healthcare, Seattle, WA, USA). The operator positioned the probe through the intercostal spaces using real-time B-mode imaging to locate a 0.5 cm × 1.5 cm liver area (the superior segment [segment 7 or 8] of the right hepatic lobe) 1–2 cm below the liver surface that was free of visible ducts or vessels. Patients received nothing by mouth for at least 8 hours before the examination. Examinations were conducted in the supine position with the right arm in maximal abduction while subjects were holding their breath. All values were measured twice, and the means of the measured values were used as the results.

Statistical analysis was conducted using SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, USA) and two-sided p values < 0.05 were considered statistically significant. Data were expressed as the means ± standard deviations. The Mann Whitney U test was used to compare outcomes between controls and cirrhotic patients and between Child-Pugh B and C cirrhotic patients. To explore the correlations between levels of inflammatory cytokines, intestinal permeability ratio, and bowel wall parameters, and between liver elasticity and US findings of the bowel wall, the Spearman’s correlation coefficient test was used.

RESULTS

The mean ages of healthy control and cirrhotic patients were 36.2 ± 2.8 and 56.2 ± 1.9 years, respectively. There were eight (40%) men and 12 (60%) women in the healthy control group and 29 (72.5%) men and 11 (27.5%) women in the cirrhotic patient group. Of the cirrhotic patients, there were 17 (85%) men and three (15%) women classified as Child-Pugh B, and 12 (60%) men and eight (40%) women classified as Child-Pugh C. The most common etiology of cirrhosis was alcohol followed by the hepatitis B virus in Child-Pugh B (13 [65%] and three [15%] patients, respectively) and C (14 [70%] and four [20%] patients, respectively) patients. There were no significant differences in levels of inflammatory cytokines or intestinal permeability ratios among the etiologies of cirrhosis. The mean Child-Pugh and MELD scores of the patients were 8.2 and 12.4 in the Child-Pugh B group and 10.5 and 15.7 in the Child-Pugh C group, respectively. The baseline characteristics of patients and healthy controls are shown in Table 1.

TNF-α levels in cirrhotic patients were significantly higher than in healthy controls (22.47 vs. 13.48 pg/mL, p < 0.001). IL-10 levels also increased in cirrhotic patients compared with healthy controls (14.91 vs. 8.57 pg/mL, p = 0.019) (Fig. 1). However, there were no significant differences between the Child-Pugh B and C groups (TNF-α, 25.44 vs. 19.51 pg/mL, p = 0.117; IL-10, 16.56 vs. 13.26 pg/mL, p = 0.549) (Table 2).

The intestinal permeability ratio was significantly higher in cirrhotic patients than in healthy controls (0.219 vs. 0.017, p < 0.001), but there was no significant difference between the Child-Pugh B and C groups (TNF-α, 25.44 vs. 19.51 pg/mL, p = 0.117; IL-10, 16.56 vs. 13.26 pg/mL, p = 0.549) (Table 2).

The wall of the terminal ileum was significantly thicker in cirrhotic patients than in healthy controls (4.2 vs. 1.9 mm, p < 0.001). The ascending colon wall was significantly less elastic in cirrhotic patients compared with healthy controls (20.4 vs. 10.9 kPa, p = 0.048) (Table 3, Fig. 2). There was a significant correlation between terminal ileum wall thickness and liver elasticity (p = 0.028). However, ascending colon wall thickness was
Table 1. Baseline characteristics of cirrhotic patients (Child-Pugh B and C classifications) and control subjects

|                      | Control | Child-Pugh B | Child-Pugh C |
|----------------------|---------|--------------|--------------|
| Number of patients included | 20      | 20           | 20           |
| Age (years)          | 36.2 (30.4–41.9) | 57.0 (50.7–63.3) | 55.4 (50.1–60.6) |
| Sex (male/female)    | 8/12    | 17/3         | 12/8         |
| Etiology             | HBV -   | 3            | 4            |
|                      | HCV -   | 2            | 2            |
|                      | Alcohol | -            | 13           |
|                      | Others | -            | 2            |
|                      | Child-Pugh score | -     | 8.2 (7.8–8.6) | 10.5 (10.0–10.9) |
|                      | MELD score | -     | 12.4 (10.1–14.6) | 15.7 (12.9–18.4) |
|                      | Hemoglobin (g/dL) | 13.8 (13.2–14.4) | 11.1 (10.1–12.1) | 10.4 (9.5–11.2) |
|                      | Platelet (× 10^9/mm³) | 216 (200–233) | 91 (73–108) | 108 (73–144) |
|                      | Prothrombin time (s) | 11.3 (11.1–11.5) | 15.4 (13.9–17.0) | 18.6 (16.7–20.5) |
|                      | Creatinine (mg/dL) | 0.75 (0.66–0.83) | 0.71 (0.59–0.82) | 0.70 (0.61–0.79) |
|                      | AST (IU/L) | 18 (15–21) | 89 (60–118) | 63 (45–81) |
|                      | ALT (IU/L) | 16 (12–20) | 27 (21–33) | 19 (15–24) |
|                      | Albumin (g/dL) | 4.68 (4.54–4.82) | 3.09 (2.86–3.31) | 2.86 (2.43–2.92) |
|                      | Total bilirubin (mg/dL) | 0.80 (0.64–0.95) | 1.73 (1.26–2.21) | 3.41 (2.48–4.34) |

Except for counts, data are presented as the means with ranges in parentheses.

HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, model for end-stage liver disease; AST, aspartic acid transaminase; ALT, alanine transaminase.

### Table 2. Levels of inflammatory cytokines and intestinal permeability (IP) ratio in control subjects and cirrhotic patients

|                      | Control                  | Cirrhosis             | p-value | Child-Pugh B | Child-Pugh C | p-value |
|----------------------|--------------------------|-----------------------|---------|--------------|--------------|---------|
| IL-10 (pg/mL)        | 8.57 (1.82–15.32)        | 14.91 (8.94–20.88)    | 0.019   | 16.56 (8.61–24.51) | 13.26 (3.67–22.85) | 0.549   |
| TNF-α (pg/mL)        | 13.48 (10.64–16.31)      | 22.47 (19.18–25.77)   | 0.000   | 25.44 (20.98–29.89) | 19.51 (14.62–24.40) | 0.117   |
| IP ratio             | 0.017 (0.012–0.022)      | 0.219 (0.090–0.348)   | 0.000   | 0.181 (0.009–0.353) | 0.259 (0.046–0.472) | 0.158   |

Values are presented as the means with ranges in parentheses.

IL, interleukin; TNF, tumor necrosis factor.

Figure 1. Distribution of levels of inflammatory cytokines and the intestinal permeability (IP) ratio in control subjects and cirrhotic patients (left, healthy controls; right, cirrhotic patients). (A) Interleukin (IL)-10 (pg/mL). (B) Tumor necrosis factor (TNF)-α (pg/mL). (C) IP ratio.
Table 3. Bowel wall parameters in control subjects and cirrhotic patients

|                     | Control     | Cirrhosis   | p-value |
|---------------------|-------------|-------------|---------|
| Ascending colon hickness (mm) | 24.2 (22.6–25.7) | 26.7 (14.0–39.5) | 0.221   |
| Ascending colon elasticity (kPa) | 10.9 (7.8–13.9) | 20.4 (5.3–35.4) | 0.048   |
| Terminal ileum thickness (mm)   | 1.9 (1.5–2.3)  | 4.2 (3.5–4.9)  | < 0.001 |
| Terminal ileum elasticity (kPa) | 11.4 (8.1–14.6) | 15.9 (6.9–24.8) | 0.133   |

Figure 2. Distribution of the thickness and elasticity of the ascending colon and terminal ileum in control subjects and cirrhotic patients (left, healthy controls; right, cirrhotic patients). (A) Thickness of the ascending colon (mm). (B) Elasticity of the ascending colon (kPa). (C) Thickness of the terminal ileum (mm). (D) Elasticity of the terminal ileum (kPa).
Table 4. Coefficients (r) of correlations between bowel wall parameters and levels of inflammatory cytokines and the intestinal permeability (IP) ratio in cirrhotic patients

|                                | IL-10 p-value | TNF-α p-value | IP ratio p-value |
|--------------------------------|---------------|---------------|-----------------|
| Ascending colon thickness      | -0.08         | 0.14          | -0.27           | 0.221           |
| Ascending colon elasticity     | 0.26          | 0.30          | -0.23           | 0.334           |
| Terminal ileum thickness       | -0.01         | 0.28          | 0.07            | 0.739           |
| Terminal ileum elasticity      | 0.07          | 0.12          | -0.06           | 0.806           |

IL, interleukin; TNF, tumor necrosis factor.

not correlated with liver elasticity (p = 0.051). The wall elasticity of the terminal ileum and ascending colon was not correlated with liver elasticity (p = 0.876 and 0.468 for the ascending colon and terminal ileum, respectively). Furthermore, there were no significant relationships between bowel wall thickness or elasticity (terminal ileum or ascending colon) and intestinal permeability or levels of TNF-α and IL-10 (Table 4).

DISCUSSION

In our study, intestinal permeability increased in cirrhotic patients compared with healthy controls. However, there was no association between disease severity and intestinal permeability. Intestinal permeability is an indicator of the gut barrier system, and when the gut barrier system is damaged, intestinal permeability increases and toxic materials such as endotoxins can invade the intestines. Previous reports have explored intestinal permeability in cirrhotic patients. Choi et al. [13] reported that intestinal permeability was higher in 27 cirrhotic patients than in the normal population, and intestinal permeability increased proportionally with degree of cirrhosis. Conversely, Benjamin et al. [14] also reported that intestinal permeability was elevated in cirrhotic patients, but the increase in intestinal permeability was not associated with degree of cirrhosis. Increased intestinal permeability is known as leaky gut syndrome [15]. Previous studies have explored therapeutic approaches to treat leaky guts in cirrhotic patients [16,17]. Antibiotics, probiotics, symbiotics, prebiotics, and combinations of these may improve the clinical course of cirrhotic patients. In addition, adequate management of the gut-liver axis may prevent liver cirrhosis by inhibiting the progression of fibrosis.

In this study, cirrhotic patients exhibited thickened bowel walls and decreased elasticity of the intestine compared with healthy controls. There was also a significant correlation between wall thickness of the terminal ileum and liver elasticity in cirrhotic patients. Portal pressure is increased due to cirrhotic changes in the liver, and venous pooling in the intestine may lead to the thickening and decreased elasticity of the bowel wall, which ultimately causes increased permeability [9]. Measuring bowel wall thickness and elasticity can help with assessing the intestinal permeability of patients and predicting the prognosis of cirrhosis. Cirrhotic patients with thickened terminal ileum walls are considered to have advanced liver fibrosis.

Karahan et al. [7] explored gastrointestinal wall thickening in cirrhotic patients using contrast-enhanced CT. The thickened bowel wall was more commonly detected in the jejunum than in the duodenum or ileum in the small intestine. We measured bowel wall thickness at the terminal ileum in the small intestine because of the poor sonic window in the jejunum and duodenum. We observed significant wall thickening in the patient group. In the colon, wall thickening was more commonly observed in the ascending colon in contrast-enhanced CT. Our study showed no significant change in wall thickness in the ascending colon compared with the control group. This difference may have resulted from the small population of enrolled patients in our study and different characteristics between patient groups between both studies.

There are potential limitations in this study. First, our results do not strongly support that elevated levels of inflammatory cytokines, increased intestinal permeability, and changes in US elastographic findings of the bowel wall are associated with complications in patients with cirrhosis. Nevertheless, associa-
tions with specific complications of cirrhosis should be investigated in the future. Second, the number of enrolled patients was small and the age discrepancy between healthy controls and cirrhotic patients was large (mean ages were 36.2 and 56.2 years for healthy controls and cirrhotic patients, respectively). Several reports have shown that human aging affects the gut barrier system and intestinal permeability [18,19]. Thus, further studies are required with a larger number of age-matched participants.

In conclusion, we observed increased intestinal permeability and levels of inflammatory cytokines in cirrhotic patients. In addition, increased wall thickness and decreased elasticity in the bowel were determined using US in cirrhotic patients. To the best of our knowledge, this is the first study to assess bowel wall changes using US in cirrhotic patients. However, we could not identify differences in these parameters according to degree of cirrhosis. No correlations between liver elasticity and bowel wall thickness or intestinal permeability were observed in our study. Further studies are required to analyze the correlations between intestinal permeability, cytokine levels, and degree of cirrhosis. To further explore the relationship between bowel wall changes and intestinal permeability, cytokine levels should be measured in cirrhotic patients.

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