Increased prevalence of intestinal inflammation in patients with liver cirrhosis

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Abstract

AIM To investigate the pathophysiology of the digestive tract in patients with liver cirrhosis.
METHODS In 42 cirrhotic patients and 20 control subjects, the following fecal proteins were measured by enzyme-linked immunosorbent assay: albumin (Alb), transferrin (Tf), and α_1-antitrypsin (α_1-AT) as a marker for intestinal protein loss, hemoglobin (Hb) for bleeding, PMN-elastase for intestinal inflammation, and secretory IgA for intestinal immunity.
RESULTS The fecal concentrations of Hb, Alb, Tf, α_1-AT, an d PMN-elastase were increased in 13 (31%), 8(19%), 10(24%), 6(14%), and 11 (26%) cases among 42 patients, respectively. Fecal concentration of secretory IgA was decreased in 7 (17%) of 42 patients. However, these fecal concentrations were not related to the severity or etiology of liver cirrhosis. The serum Alb level was significantly decreased in patients with intestinal protein loss compared to that in patients without intestinal protein loss.
CONCLUSION These findings suggest that: ① besides the well- known pathological conditions, such as bleeding and protein loss, intestinal inflammation and decreased intestinal immunity are found in cirrhotic patients; ② intestinal protein loss contributes to hypoalbuminemia in cirrhotic patients, and ③ intestinal inflammation should not be over looked in cirrhotic patients, si nce it may contribute to or cause intestinal protein loss and other various path ological conditions.

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INTRODUCTION

Gastrointestinal symptoms are common in patients with liver cirrhosis and portal hypertension. Their pathophysiology remains, for the most part, obscure. It is well known that esophageal varices and portal hypertensive gastropathy[1,2] are common causes of upper gastrointestinal (GI) bleeding in patients with liver cirrhosis. Recently, portal hypertensive colopathy, such as colonic vascular ectasia and rectal varices, were recognized as causes of lower gastrointestinal bleeding in cirrhotic patients[3,4]. Other than bleeding, various pathological conditions such as malabsorption[5,6] and intestinal protein loss[7,8] have been reported in cirrhotic patients. We developed fecal tests that are useful for evaluating the various pathophysiologies of the digestive tract[9-12]. Fecal hemoglobin (Hb) is a useful marker of bleeding. Fecal albumin, transferrin (Tf), and α_1-antitrypsin (α_1-AT) are useful markers of intestinal protein loss. Fecal secretory IgA (sIgA) level reflects the condition of local immunity in the intestine and an increase in fecal neutrophil granule-derived proteins, such as PMN-elastase, indicates the presence of intestinal inflammation. In the present study, we examined the fecal protein profile to investigate the pathophysiology of the digestive tract in cirrhotic patients.

MATERIALS AND METHODS

Subjects

Forty-two patients with liver cirrhosis aged 59.0 ± 8.7 years (mean $\pm$ SD) were evaluated. The diagnosis of liver cirrhosis in each case was confirmed by a comb ination of clinical, biochemical, radiological, and pathological methods. The clinical severity of the liver disease in each case was determined using the Pugh modification of Child's original classification[13]. These cases consisted of thirty-one males and eleven females. The number of patients with Child A, Child B, and Child C were 27, 6, and 9, respectively. The etiology of the liver cirrhosis was as follows: 33 patients with hepatitis C, 3 with hepatitis B, and 6 with alcoholism. All patients had

no obvious gastrointestinal bleeding. The control group consisted of 20 subjects aged 41.0±18.1 years with no demonstrated abnormality in the upper or lower digestive tract. Informed consent was obtained from each subject in accordance with the Helsinki Declaration.

Stool collection and measurement of fecal proteins

Patients were instructed to defecate into a polystyrene container (diameter 15 cm, depth 12 cm). The stool samples, collected at 4 ℃ over a period of 48 h - 72 h, were homogenized with a small amount of water, then sto red at -80 ℃ until the time of measurement. The concentrations of fecal Hb, Alb, Tf, α_1-AT, PMN-elastase, and sIgA were measured by enzyme -linked immunosorbent assay (ELISA) as described previously[9-11]. Brie fly, anti-human Hb antibody (Institute of Immunology, Japan), anti-human albumin antibody (Dakopatts, Glostrup, Denmark), anti-human Tf anti body (Dakopatts), anti-human α_1-AT antibody (Dakopatts), anti-human PMN-elastase antibody (Serotec, Oxford, England), or anti-human secretory component antibody (Dakopatts) was coated in the wells of a 96-well microplate. The diluted fecal samples (100 to 10000 fold) were added to each well. After reaction at 37 ℃ for 1 h, the wells were washed with water . The samples were then reacted with the respective alkaline phosphatase- labeled antibody except alkaline phosphatase labeled anti-human IgA antibody (Dakopa tts) for ELISA of sIgA. The enzyme reaction was then carried out, and color deve lopment was measured with a microplate colorimeter at 510/630 nm.

Statistical analysis

Values were expressed as medians (25%, 75%). The Mann-Whitney U tests and/or χ^2 tests were used to compare groups. All *P* values were two-tailed; *P* values less than 0.05 were considered statistically significant.

RESULTS

Fecal protein concentrations in control subjects

Results are shown in Figure 1. Daily stool weight (g/day) was 140 (100, 230) in control subjects. The concentrations of fecal Hb, Alb, Tf, α_1-AT, sIgA, and PMN-elastase were 1.5 (0.1, 4.0), 0.1 (0.1, 1.8), 0.1 (0.1, 0.4), 327.4 (201.1, 421.3), 214.9 (56.6, 244.1), and 0.6 (0.3, 1.2), respectively. The 95th percentile of the control

subjects was used as the cu t-off value (10.6, 5.3, 1.0, 771, and 2.2 μg/g for Hb, Alb, Tf, α_1-AT, and PMN elastase, respectively). sIgA was important when not only increasing, but also decreasing. Therefore, the lower limit of the normal range was defined as the 5th percentile (26.8 μg/g) of the control subjects for sIgA.

Fecal protein concentrations in cirrhotic patients

Daily stool weight (g/day) was 140 (100, 180). In terms of daily stool weight, there was no significant difference between the cirrhotic patients and control subjects. As shown in Figure 1, the fecal concentrations of Hb, Alb, Tf, α_1-AT, and PMN-elastase were increased in 13 (31%), 8(19%), 10 (24%), 6(14%), and 11 (26%) among 42 patients, respectively. Conversely, the fecal con centration of sIgA was decreased in 7 (17%) of 42 patients. There were significa nt differences in fecal Hb, Alb, sIgA, and PMN-elastase concentrations between the cirrhotic patients and the controls.

Relationship between fecal protein concentrations and the severity of liver cirrhosis

As shown in Figure 2, there was no relationship between the concentrations of these fecal proteins and the severity of the disease.

Relationship between fecal protein concentrations and the presence of esophageal varices

As shown in Figure 3, fecal Hb concentration demonstrated a significant difference between the cirrhotic patients and the controls.

Relationship between fecal protein concentrations and the etiology of liver cirrhosis

There was no association between fecal protein concentrations and the etiology of the liver disease (data not shown).

Intestinal protein loss and nutritional status in cirrhotic patients

Results are shown in Figure 4. The patient was defined as having intesti nal protein loss, when a patient had increased concentrations of at least one of Alb, Tf, and α_1-AT. The serum Alb level was significantly decre ased in patients with intestinal protein loss compared to that in patients without intestinal protein loss. The findings suggest that intestinal protein loss contribute to the development of hypoalbuminemia in cirrhotic patients.
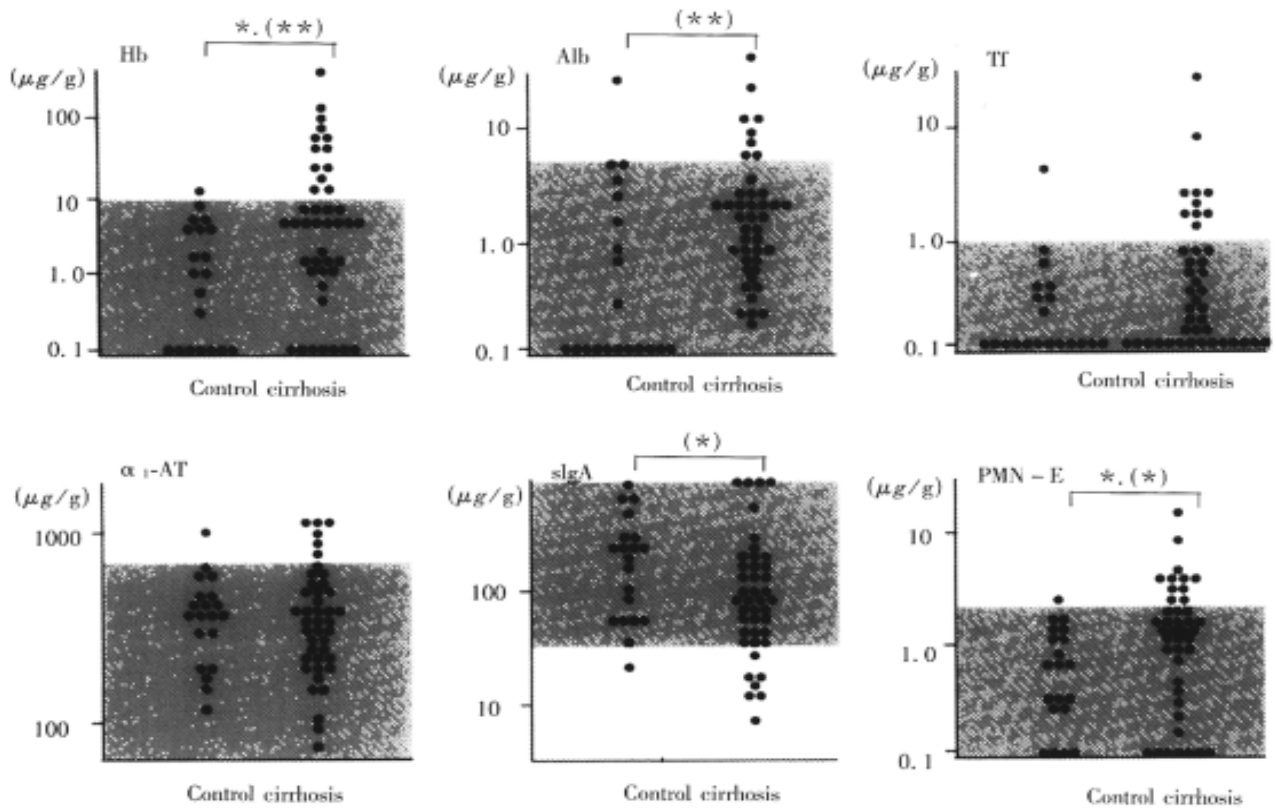
Figure1 Fecal protein concentrations in cirrhotic patients and control subjects. The dotted area shows the 95 percentile of the c ontrol subjects. [a]: *P*<0.05 by c[2] test; [b]: *P*<0.05 ; [c]:*P*<0.01 by Mann-Whitney U test.
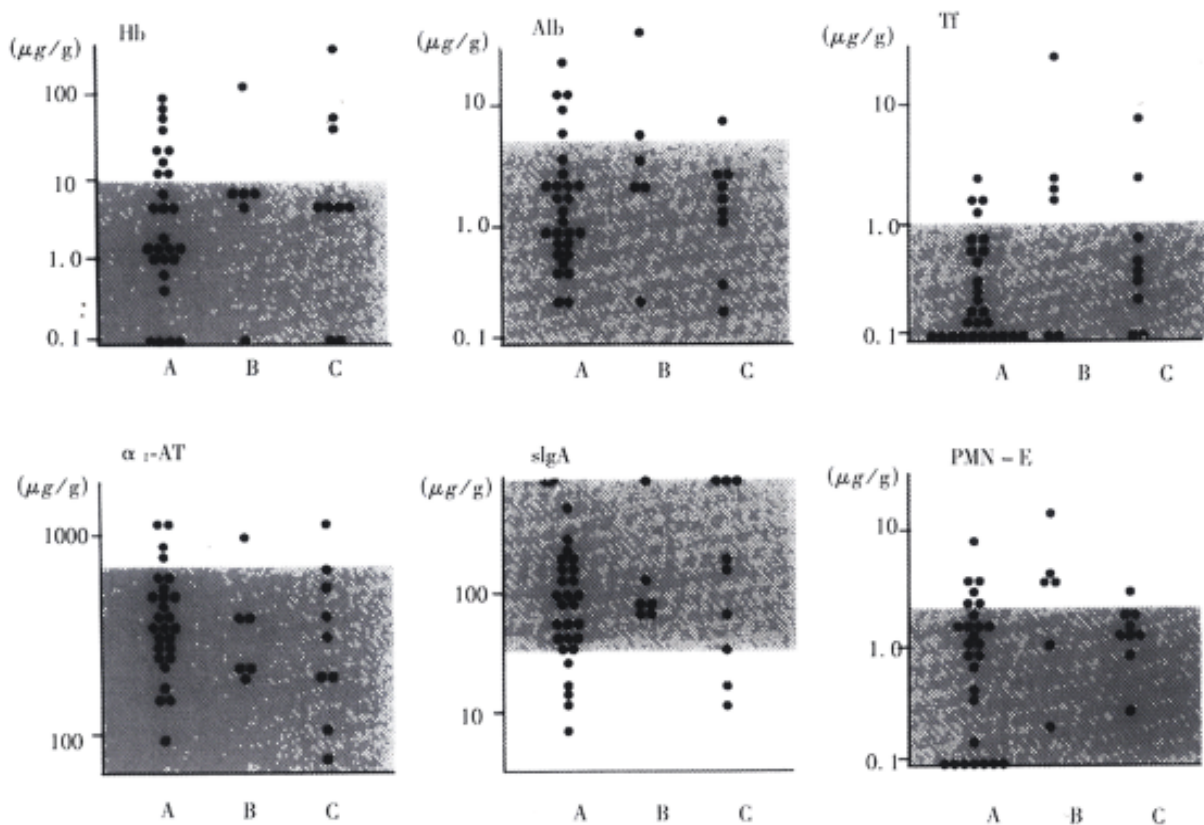

Figure2 Relationship between fecal protein concentr ations and the severity of the liver cirrhosis. A: Child A, B: Child B, C: Child C. The dotted area shows the 95 percentile of the control subjects.
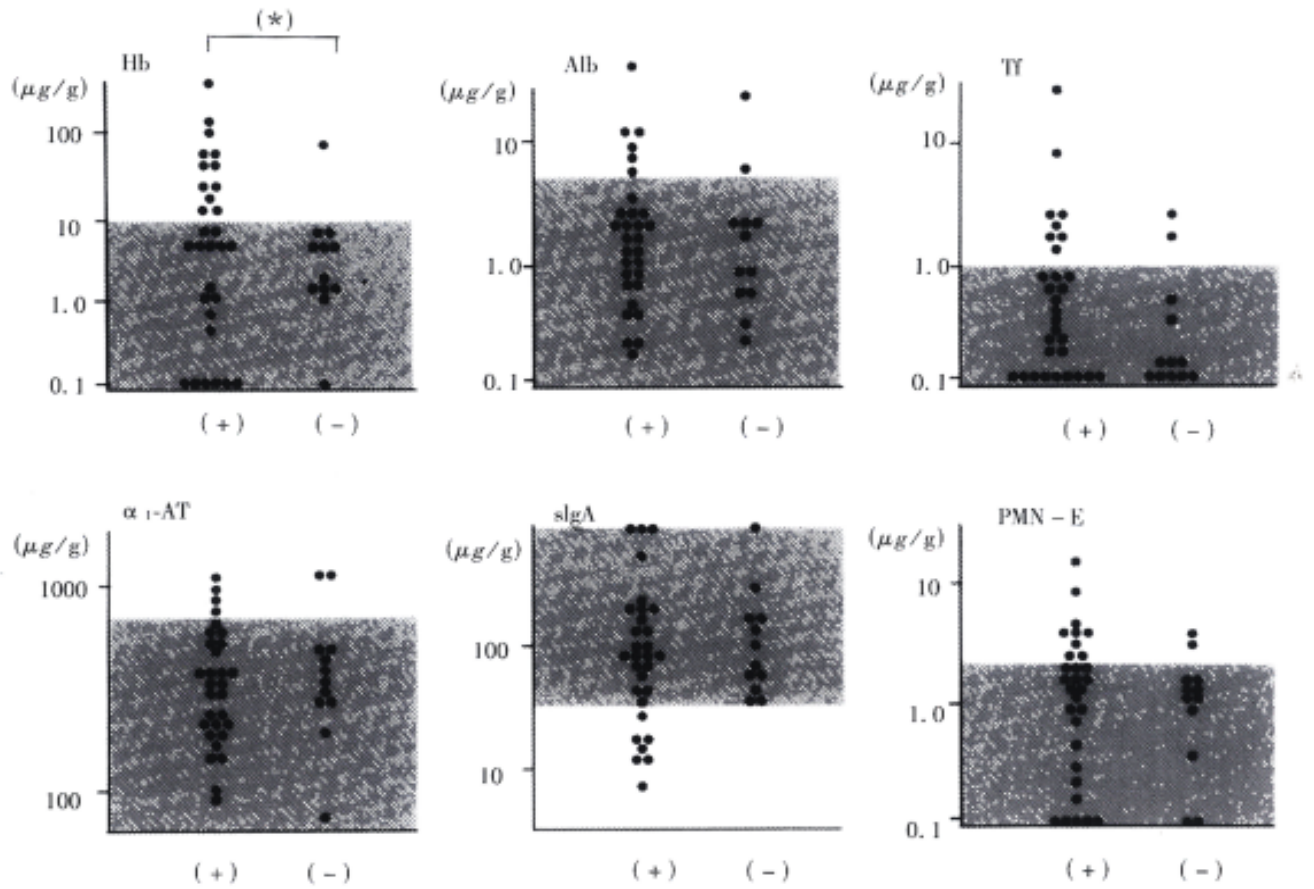
Figure3 Relationship between fecal protein concentr ations and the presence of esophageal varices. (+): cirrhotic patients associate d with esophageal varices; (-): cirrhotic patients not associated with esophagea l varices; [a]: $P<0.05$ by X^2 test.
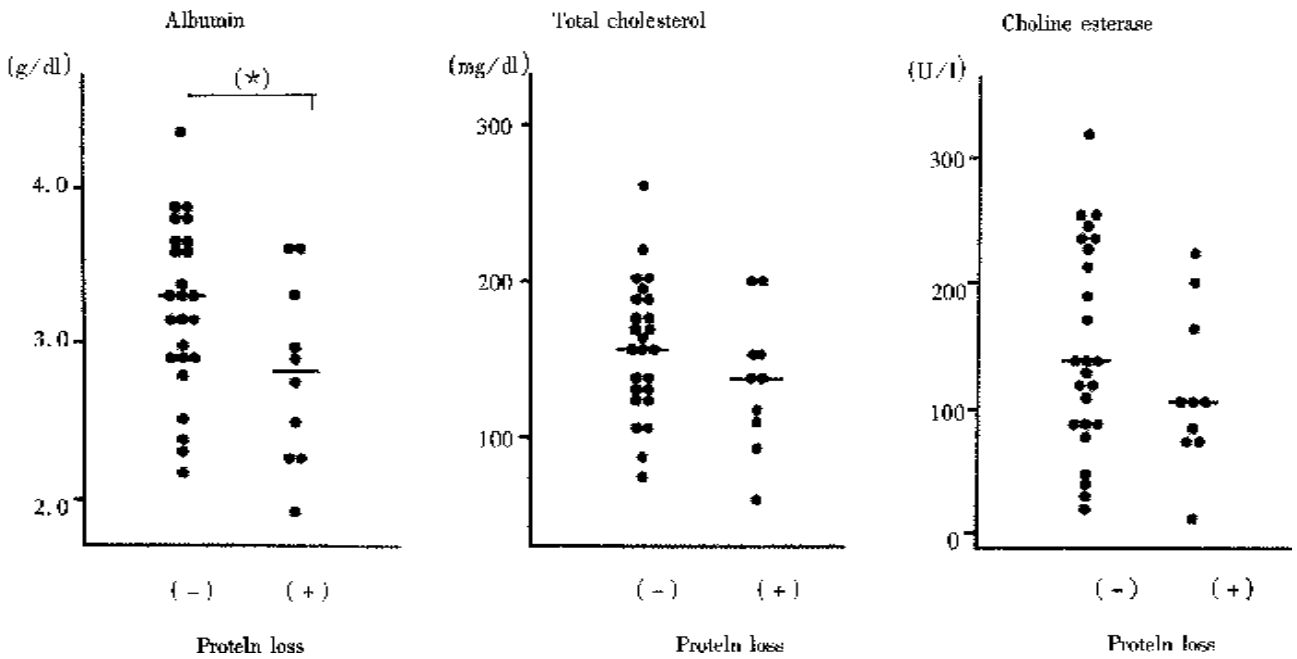

Figure4 Serum concentrations of albumin, total chol esterol, and choline esterase in cirrhotic patients. Comparison between groups with or without intestinal protein loss. [a]: $P<0.05$, NS; not significa nt by Mann-Whitney U test.
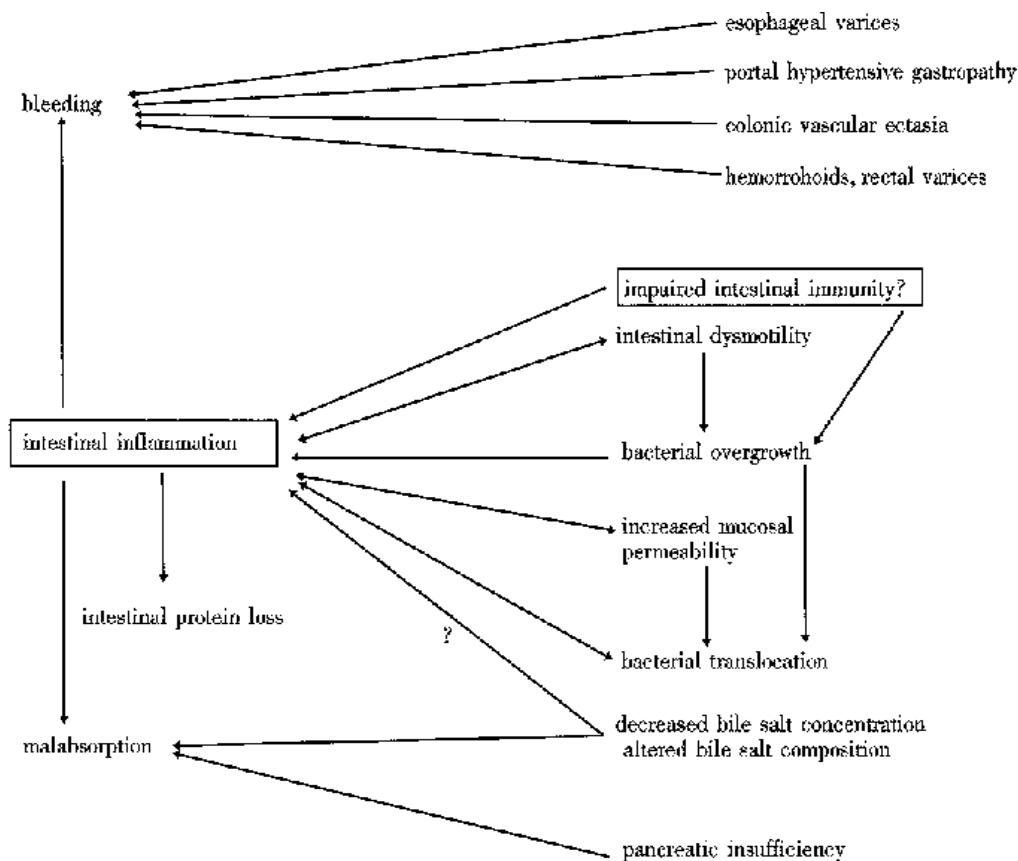
Figure5 Proposed pathophysiology of digestive tract in cirrhotic patients.

DISCUSSION

The present study showed that 26% of the cirrhotic patients had elevated fecal PMN-elastase concentrations and suggested an increased prevalence of intestinal inflammation in cirrhotic patients. Furthermore, occult GI bleeding and intestin alprotein loss were observed commonly even in cirrhotic patients who had no mas sive GI bleeding. The conditions such as bleeding, intestinal protein loss, impa ired intestinal immunity, and intestinal inflammation can be considered to be included in portal hypertensive enteropathy, because these seemed to be related not to the severity of the liver disease, but to portal hypertension. Recently, Stan ley *et al*[14] first demonstrated that many of the manifestations of portal hypertensive enteropathy can be corrected by a trasjugular intrahepatic portosystemic stent-shunt (TIPS) procedure. They described a cirrhotic patient with diarrhea and hypoalbuminemia. Protein losing enteropathy was confirmed by analysis of whole gut lavage fluid. They then performed a TIPS procedure, which eliminated the portal hypertension. Both the patient's diarrhea and elevated whole gut lavage fluid protein concentrations were reduced.

It is well known that the mechanism of hypoalbuminemia observed in cirrhotic patients is multifactorial. Decreased albumin production in the liver is one of the causes. In the present study, the serum Alb level was decreased significantly in patients with intestinal protein loss compared to that in patients without intestinal protein loss. The findings suggest that intestinal protein loss contributes to the development of hypoalbuminemia in cirrhotic patients.

Little attention has been paid to the relation between liver cirrhosis and intestinal inflammation. The prevalence of colitis diagnosed by colonoscopy in cirrhotic patients is not well established in the literature, and ranges from 10% to 57.9%[3,15-18]. Such a wide distribution is considered to be due to diff erences in the definition of inflammation and wide variation between observers in describing the mucosal appearance. The highest value, 57.9%, was reported by Scandalis N *et al*[16]. In their study, inflammatory changes included mucosal edema as well as erythema, granularity, and fragility of the mucosa. In contrast to colonoscopy, stool test is presumably a relatively objective test for assessment of inflammation. The value obtained in the present study (26%) was highest among the reported prevalence except for 57.9% reported by Scandalis N *et al*. The following possible reasons

should be taken into consideration. ① Fecal PMN-elastase, a marker of intestinal inflammation in the present study, reflects mucosal inflammation of the small intestine as well as the colon. The prevalence of inflammation of the small intestine has not been studied so far. ② Difference in sensitivity between the stool test and colonoscopy: the stool test seems to be more sensitive than colonoscopy, since colonoscopy does not always detect inflammation if the inflammation is minimal or localized.

Our proposed pathology of the digestive tract in cirrhotic patients is shown in Figure 5. The present study suggested that impaired intestinal immunity might be one of the predisposing factors to intestinal inflammation. In the present study, 17% of the cirrhotic patients showed decreased fecal sIgA concentrations. This is the first report concerning fecal sIgA in cirrhotic patients, although serum sIgA in cirrhotic patients was shown previously to be elevated[19]. Besides impaired intestinal immunity, predisposing factors for intestinal inflammation include intestinal dysmotility, bacterial overgrowth, bacterial translocation, and increased mucosal permeability[20]. Recently, we reported that bile acids inhibit tumor necrosis factor-α-induced interleukin-8 production in human intestinal cells[21]. A decreased level or an altered composition of the intraluminal bile acids may make cirrhotic patients suscept ible to intestinal inflammation[22,23].

It is well known that GI bleeding, intestinal protein loss, malabsorption, and bacterial translocation develop in patients with inflammatory bowel diseases[10,24,25]. In cirrhotic patients with intestinal inflammation, therefore, such serious pathological conditions may easily occur. Therefore, it is importa nt to evaluate whether a cirrhotic patient has intestinal inflammation or not in order to manage the patient. Measurement of fecal PMN-elastase is preferable to colonoscopy as a screening test for intestinal inflammation in cirrhotic patients, since the former is a sensitive and non-invasive test.

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