Increased intestinal macromolecular permeability and urine nitrite excretion associated with liver cirrhosis with ascites

Soong Lee, Seung-Cheol Son, Moon-Jong Han, Woo-Jin Kim, Soo-Hyun Kim, Hye-Ran Kim, Woo-Kyu Jeon, Ki-Hong Park, Myung-Geun Shin

Myung-Geun Shin, Soo-Hyun Kim, Department of Laboratory Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun 519-809, Korea
Soong Lee, Seung-Cheol Son, Moon-Jong Han, Woo-Jin Kim, Department of Internal Medicine, College of Medicine, Seonam University and Seonam University Hospital, Gwangju 502-157, Korea
Hye-Ran Kim, Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University Medical School, Gwangju 501-757, Korea
Woo-Kyu Jeon, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul 110-746, Korea
Ki-Hong Park, Korea Polymer Testing & Research Institute Ltd., Seoul 136-120, Korea
Author contributions: Lee S, Son SC, Han MJ, and Kim WJ designed research and analyzed data; Kim SH, Kim HR, Jeon WK, and Park KH performed research; Shin MG contributed to interpretation of the data and critical revision of the manuscript.
Supported by: A grant from the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea, No.0520190-1
Correspondence to: Myung-Geun Shin, MD, PhD, Department of Laboratory Medicine, Chonnam National University Medical School and Chonnam National University Hwasun Hospital, Hwasun 519-809, Korea. mgshin@chonnam.ac.kr
Telephone: +82-61-3797950 Fax: +82-61-3797984
Received: March 24, 2008 Revised: May 19, 2008
Accepted: May 26, 2008
Published online: June 28, 2008

Abstract

AIM: To determine intestinal permeability, the serum tumor necrosis factor (TNF)-α level and urine nitric oxide (NO) metabolites are altered in liver cirrhosis (LC) with or without ascites.

METHODS: Fifty-three patients with LC and 26 healthy control subjects were enrolled in the study. The intestinal permeability value is expressed as the percentage of polyethylene glycol (PEG) 400 and 3350 retrieval in 8-h urine samples as determined by high performance liquid chromatography. Serum TNF-α concentrations and urine NO metabolites were determined using an enzyme-linked immunosorbent assay (ELISA) and Greiss reaction method, respectively.

RESULTS: The intestinal permeability index was significantly higher in patients with LC with ascites than in healthy control subjects or patients with LC without ascites (0.88 ± 0.12 vs 0.52 ± 0.05 or 0.53 ± 0.03, P < 0.05) and correlated with urine nitrite excretion (r = 0.98). Interestingly, the serum TNF-α concentration was significantly higher in LC without ascites than in control subjects or in LC with ascites (198.9 ± 55.8 pg/mL vs 40.9 ± 12.3 pg/mL or 32.1 ± 13.3 pg/mL, P < 0.05). Urine nitrite excretion was significantly higher in LC with ascites than in the control subjects or in LC without ascites (1170.9 ± 28.7 μmol/L vs 903.1 ± 55.1 μmol/L or 956.7 ± 47.7 μmol/L, P < 0.05).

CONCLUSION: Increased intestinal macromolecular permeability and NO is probably of importance in the pathophysiology and progression of LC with ascites, but the serum TNF-α concentration was not related to LC with ascites.

© 2008 The WJG Press. All rights reserved.

Key words: Intestinal permeability; Tumor necrosis factor-α; Nitric oxide; Liver cirrhosis; Ascites

Peer reviewer: Dr. Soren Moller, Department of Clinical Physiology 239, Hvidovre Hospital, Kettegaarde allé 30, DK-2650 Hvidovre DK-2650, Denmark

Lee S, Son SC, Han MJ, Kim WJ, Kim SH, Kim HR, Jeon WK, Park KH, Shin MG. Increased intestinal macromolecular permeability and urine nitric oxide excretion associated with liver cirrhosis with ascites. World J Gastroenterol 2008; 14(24): 3884-3890 Available from: URL: http://www.wjgnet.com/1007-9327/14/3884.asp DOI: http://dx.doi.org/10.3748/wjg.14.3884

INTRODUCTION

It has been shown that the gut, as a reservoir of enteric bacteria in the body, plays a protective role as mucosal barrier function, immunoglobulin secretion, and local and systemic macrophage system, but under liver cirrhosis (LC) with portal hypertension a correlative connection between liver damage and the functional activity of the intestine with mucosal abnormalities exist[1-3]. Increased intestinal permeability (IPI) with bacterial translocation and endotoxemia have been implicated...
in the pathogenesis of chronic liver injury and as contributory factors in the development of dangerous complications, such as encephalopathy and bacterial infections in LC. However, other investigators have suggested that intestinal permeability is probably of limited importance in the pathophysiology of bacterial infections in patients with LC. Intestinal permeability in LC has been reported as being increased or normal. The development of systemic endotoxemia may in turn act through the release of cytokines, to further increase intestinal permeability, impair host immunity and promote bacterial translocation from the gut, thus resulting in a vicious circle. It has been proposed that some of these cytokines play a role in several known cirrhosis-related complications, such as hyperdynamic circulation, susceptibility to infection, and hepatic encephalopathy. Tumor necrosis factor (TNF-α) is a 17 kDa cytotoxic protein produced by mononuclear cells on activation by bacterial endotoxin and tissue injury. However, the TNF-α level in LC has been reported with controversial findings, as it may or may not correlate with an advanced stage of disease and a worse outcome.

Nitric oxide (NO) has a role in cirrhosis. Endotoxemia, possibly from gut-derived bacterial translocation, causes induction of NO synthase leading to increase vascular NO production, which is the primary stimulus for the development of vasodilatation in cirrhosis and its accompanying clinical manifestations. While NO is an unstable molecule, one means of investigating NO formation is to measure nitrite (NO₂⁻), which is one of two primary stable non-volatile breakdown products of NO. A dose dependent increase in nitrite has been demonstrated to occur when macrophages are activated with lipopolysaccharide (LPS) both in vitro and in vivo.

Limited data exists on the state of intestinal macromolecular permeability using polyethylene glycol (PEG) (400 and 3350) in cirrhotic patients with or without ascites. To clarify the role of intestinal macromolecular permeability, the serum TNF-α level and nitrite level in urine to the development of LC with ascites, we investigated whether intestinal macromolecular permeability is altered in patients LC with or without ascites, and its relationship with the serum TNF-α level and NO metabolite level in urine.

**Materials and Methods**

**Patients and healthy control subjects**

Participating patients and healthy control subjects were comprised of 26 patients with LC with ascites, 27 patients with LC without ascites and 26 age and sex-matched healthy individuals with a normal medical history, physical examination and blood chemistry. Subjects with known infection, gastrointestinal or renal disease or diabetes mellitus were excluded from the study. Also excluded were patients that received substances known to affect intestinal permeability test results such as lactulose, non-steroidal anti-inflammatory drugs, or alcohol, in the previous 2 wk. The Institutional Review Board of the Seonam University Health Sciences Center (Namwon, Korea) approved the study. All subjects in this study gave informed consent. The diagnosis of LC was based on the typical findings of hepatic cirrhotic appearance, splenomegaly, esophageal varices, and ascites by ultrasonography and an upper gastrointestinal endoscopy, and laboratory results (prolonged prothrombin time, hypoalbuminemia with or without elevated liver enzymes). The severity of liver disease was determined according to Child-Pugh criteria.

**Measurement of intestinal macromolecular permeability**

Urine samples used in this study were collected during 8 h from subjects that fasted overnight (last meal before 8 PM the day before). Subjects ingested a 100 mL test solution containing 1 g of PEG 400 and 10 g of PEG 3350 in water. Each subject ingested the PEG solution 1 h before a breakfast meal. Urine samples were been kept frozen (-20°C) until processing for analysis. In this study, we attempted to detect PEG 400 as a low molecular weight (MW) marker and 3350 as a higher MW marker simultaneously in urine samples by high performance liquid chromatography (HPLC) using evaporative light scattering detection. About 2 mL of urine was filtered through a 0.45 μm syringe filter (Nylon membrane) and stored at 4°C until analysis. All of the 1 mL-vidal urine samples for analysis were directly placed into a Waters 717+ autosampler with refrigerator (10°C). The HPLC column was a 5 μm PLRP-S 100 A column (150 mm × 4.6 mm, Polymer Laboratories, Amherst, MA USA) packed with PS/DVB polymeric beads. To remove particles in the urine sample, a disposable Security Guard kit (Phenomenex, Torrance, CA USA) was used with the HPLC column. A gradient mobile phase (acetonitrile/H₂O) for an elution of 40-60 min was used to separate efficiently all hydrophilic and hydrophobic compounds. As the HPLC clients were controlled by a gradient controller program, we tried to set the program to allow the impurities elute first while the marker compounds (PEG 400 and PEG 3350) eluted later without peak overlap. The eluted components were analyzed by an evaporative light scattering detector (PL-ELSD 2100 under conditions of vaporization -85°C, nebulizer 85°C and gas flow 1.0; Polymer Laboratories). Calibration curves were obtained in the range of 200-1500 mg/L for PEG 400 and 10-200 mg/L for PEG 3350, respectively. The intestinal permeability was calculated by the concentration of the PEG marker compound and total urine volume. The calculated intestinal permeability index (IPI, in %), PEG retrieval ratio, is an expression of the PEG 3350 intestinal permeability, relative to PEG 400.
Table 1  Demographics and characteristics of the subjects
(mean ± SE)

|                          | Cirrhotics with asites (n = 26) | Cirrhotics without asites (n = 27) | Healthy controls (n = 26) |
|--------------------------|--------------------------------|-----------------------------------|--------------------------|
| Age (yr)                 | 54.7 ± 9.6                     | 53.9 ± 9.7                        | 50.3 ± 9.2               |
| Sex (M/F)                | 25/3                           | 21/6                              | 17/9                     |
| Etiology                 |                               |                                   |                          |
| Alcohol                  | 16                             | 15                                |                          |
| Viral†                   | 9                              | 12                                |                          |
| Alcohol/viral†           | 1                              | 0                                 |                          |
| Child class (A/B/C)      | 1/16/9                         | 22/5/0                            |                          |
| Child-Pugh score         | 8.8 ± 0.44a                    | 6.3 ± 0.34                        |                          |
| Serum albumin (g/dL)     | 2.8 ± 0.11b                    | 3.5 ± 0.12                        |                          |
| Serum bilirubin (mg/dL)  | 5.1 ± 0.92c                    | 2.0 ± 0.43                        |                          |
| Prothrombin time (s)     | 15.7 ± 0.50a                   | 14.3 ± 0.40                       |                          |
| AST (IU/L)               | 78.3 ± 9.45                    | 74.8 ± 11.4                       |                          |
| ALT (IU/L)               | 34.1 ± 4.8                     | 51.5 ± 8.9                       |                          |
| Encephalopathy           | 9b                             | 2                                 |                          |
| Esophageal varix         | 11                             | 9                                |                          |

†Viral etiology—cirrhosis with asites (HBV-6, HCV-3) and cirrhosis without asites (HBV-9, HCV-4); †Viral etiology—HBV-1. *P < 0.05; **P < 0.01 vs cirrhosis without asites.

Measurement of serum TNF-α

With in a 12 h period after oral administration of the PEG solution, 10 milliliters of a blood sample was taken from a forearm vein of each individual. All blood samples were anticogulated with EDTA and then plasma was separated by centrifugation at 1600 g for 15 min. Plasma samples were stored at -70°C until analysis. The serum TNF-α concentration was determined by the enzyme-linked immunosorbent assay (ELISA) technique (Quantikine® human TNF-α, R & D Systems, Minneapolis, MN USA), according to the manufacturer instructions.

Measurement of urinary nitrite excretion

About 2 mL of urine was filtered through a 10000 MW filter (Millipore Microcon YM-10) and was assayed for the NO metabolite nitrite by the Greiss reaction using Parameter TM Total NO/Nitrite/Nitrate kit (R &D Systems). The total concentration of nitrite was determined by absorbance at 540 nm after urine nitrate (NO₃⁻) was converted to nitrite (NO₂⁻) by the NADPH-dependent nitrate reductase.

Statistical analysis

Data are reported as mean values and standard errors (mean ± SE) or percentage according to variables. Differences among the three groups were analyzed by ANOVA. When a significant effect occurred, Scheffe post hoc comparisons were used to test differences among the means. An independent samples t-test was used to compare test results between two groups. A nonparametric test, the Mann-Whitney test, was used to compare independently two groups that had fewer than 10 samples. We calculated Pearson’s correlation coefficient for associations between two variables. SPSS statistical software (version 11.0) was used for the statistical analysis. A two-tailed significant level of 5% was chosen as a type I error.

RESULTS

Characteristics of the participating patients

There were no significant differences regarding age and gender between the cirrhotic patients with or without ascites and healthy control subjects. The distribution of causes of LC were alcohol (n = 31), viral infection (HBV 14, HCV 7; n = 21) and alcohol combined with HBV infection (n = 1). Renal function as assessed based on blood urea and creatinine levels was normal in all patients and control subjects. Details of the demographics, etiology, severity, complications of the LC and concurrent infections are outlined in Table 1.

Intestinal macromolecular permeability

Mean values for PEG 400 and 3350 retrieval were 46.5 ± 3.22 and 0.24 ± 0.03 in control subjects, 44.1 ± 5.17 and 0.21 ± 0.02 in patients with LC without asites and 37.4 ± 3.55 and 0.31 ± 0.04 in patients with LC with asites, respectively. The mean values for the IPI were different in patients from the healthy control subjects and patients with LC without ascites reflected the expected low diffusion of PEG 3350, being significantly higher in patients with LC with asites (0.52 ± 0.05 and 0.53 ± 0.03 vs 0.88 ± 0.12, P < 0.05) (Figure 1). However, there was no significant difference between the healthy control subjects and patients with LC without asites (Table 2).

A sub-analysis relating intestinal permeability to the severity of LC for all patients as indicated by the Child-Pugh class showed significant differences between class A, B and C for PEG 3350 (0.20 ± 0.02, 0.25 ± 0.03 vs 0.42 ± 0.08, P < 0.05) and IPI (0.52 ± 0.04, 0.72 ± 0.07 vs 1.12 ± 0.27, P < 0.05). According to sub-analysis relating IPI to the presence of complications of LC for patients as indicated by encephalopathy and hypoalbuminemia, there were significant differences (P < 0.05), but not for
patients as indicated by a prolonged prothrombin time, esophageal varix or hyperbilirubinemia.

**Serum TNF-α level**

The concentration of serum TNF-α was 198.9 ± 55.8 pg/mL in patients with LC without ascites, 32.1 ± 13.3 pg/mL in LC with ascites and 40.9 ± 12.3 pg/mL in the control subjects (Figure 2). A group comparison by the Mann-Whitney test showed that the serum TNF-α level was significantly higher in patients with LC without ascites than in the control subjects and in patients with LC with ascites (P < 0.05) (Table 2). According to the sub-analysis relating the TNF-α level to the severity of LC as measured by the Child-Pugh class and to the presence of complications of LC for patients as indicated by the presence of encephalopathy, hypoalbuminemia, prolonged prothrombin time, esophageal varix and hyperbilirubinemia, there were no significant differences (Table 3).

**Urinary nitrite excretion**

Urinary nitrite excretion was significantly increased in patients with LC with ascites as compared to patients with LC without ascites or the healthy control subjects (1170.9 ± 28.7 μmol/L vs 956.7 ± 47.7 μmol/L or 903.1 ± 55.8 μmol/L, P < 0.05) (Figure 3 and Table 2). According to a sub-analysis relating urinary nitrite excretion to the severity of LC as measured by the Child-Pugh class and to the presence of complications of LC for patients as indicated by the presence of encephalopathy, hypoalbuminemia, prolonged prothrombin time, esophageal varix and hyperbilirubinemia, there were no significant differences (Table 3).

**Correlation and statistical analysis**

Patients with alcoholic versus non-alcoholic cirrhosis did not differ significantly (P > 0.05) in the intestinal macromolecular permeability (PEG 400, 3350 retrieval and IPI), serum TNF-α level and urine nitrite level. There was a positive correlation between the Child-Pugh score and increasing intestinal macromolecular permeability (r = 0.494 for PEG 3350 and r = 0.447 for IPI, P < 0.01), but no significant correlation between the Child-Pugh score and the TNF-α level or urinary nitrite level among patients with LC with or without ascites. No significant correlation was observed between PEG 400, 3350 percentage retrieval, IPI and the serum TNF-α level, between the TNF-α level and urine nitrite level but there was a significant correlation between IPI and urine nitrite excretion (r = 0.98, P < 0.05) among patients with LC with or without ascites.

**DISCUSSION**

The concept of altered intestinal permeability is important and has been implicated in a number of pathological situations, including celiac disease associated with antigen permeability, allergic intestinal diseases such as digestive hypersensitivity, inflammatory diseases such as Crohn’s disease, ulcerative colitis, acute pancreatitis, alcoholic liver disease and LC associated with substance permeability during inflammation.

The pathogenic mechanisms implicated in the failure of intestinal barrier in cirrhosis have not been fully elucidated as yet and remains to be investigated.

---

**Table 2** Intestinal permeability, serum TNF-α and urine nitrite levels in the healthy control subjects and cirrhotic patients (mean ± SE).

|                | Cirrhotics with ascites (n = 26) | Cirrhotics without ascites (n = 27) | Healthy controls (n = 26) |
|----------------|----------------------------------|-------------------------------------|--------------------------|
| PEG400         | 37.4 ± 3.55                      | 44.1 ± 5.17                         | 46.5 ± 3.22              |
| PEG3350        | 0.31 ± 0.04                      | 0.21 ± 0.02                         | 0.24 ± 0.03              |
| IPI            | 0.88 ± 0.12                      | 0.53 ± 0.03                         | 0.52 ± 0.05              |
| Nitrite        | 1170.9 ± 28.7                    | 956.7 ± 47.7                        | 903.1 ± 55.1             |
| TNF-α          | 32.1 ± 13.3                      | 198.9 ± 55.8                        | 40.9 ± 12.3              |

PEG: Polyethylene glycol; IPI: Intestinal permeability index. *P < 0.05.

---

**Figure 2** Tumor necrosis factor-α (TNF-α) levels in the healthy control subjects and the cirrhotic patients. *P < 0.05.

**Figure 3** Urinary nitrite excretion in the healthy control subjects and the cirrhotic patients. *P < 0.05.
Toxic metabolites of alcohol are known to induce alterations of enterocyte tight junctions, which may increase paracellular permeability[23]. However, other inflammatory conditions may alter barrier integrity, as measured by increased gut permeation. An alternative mechanism may be the proinflammatory cytokines, which can be produced locally by epithelial cells or may reach the intestinal mucosa from an inflammatory focus distant from the bowel[24]. Interestingly, in vitro studies in cell monolayers suggest that cytokines may mediate these permeation effects by changes in the production of NO[23]. The mechanism for this effect is not known, but may involve relaxation of the cytoskeleton or oxidation/nitration of cytoskeleton proteins[26].

In the current study, PEG with different molecular masses was used to assess gut permeability, as it combines unique attributes in its chemical structure. It is non-toxic, water-soluble, as is endotoxin, and not metabolized either by the host or by intestinal bacteria[25]. After transmigration into the blood, the polar PEG-molecule is excreted with the urine. Because of its homogeneous chemical properties, its appropriately adaptable molecular mass and its linear, chain-like shape (mimicking the comparable structure of endotoxin)[25], PEG seems to be an appropriate probe for the assessment of LPS translocation through the intestine. All of these demands cannot be met by other commonly used permeability marker compounds such as mono- or disaccharides, sugar alcohols, complexes with radioactive nuclides (51Cr-EDTA, 99mTc-DTPA), proteins, or even combinations of these compounds[26].

The simultaneous use of two test marker compounds allows the expression of global intestinal permeability as an index reflecting the transfer value of the less permeable test marker (PEG 3350) relative to the most diffusible probe (PEG 400). Since pre-absorption factors such as gastric emptying, dilution by digestive secretions and post-absorption factors such as systemic distribution and renal clearance are assumed to affect both molecules equally, the value of this index should then be directly comparable from one individual to another[20].

It has been suggested that intestinal permeability markers pass through either a transcellular or a paracellular pathway. However, it is difficult to determine that endotoxins or other bacterial toxins from the gut lumen into the portal system pass paracellularly or transcellularly in cirrhotic patients, as there was no significant difference of PEG 400 and 3350 retrieval between the control subjects and cirrhotic patients with or without ascites in this study. To address this issue, further studies are needed for morphological or molecular changes of intestinal mucosa in LC during the PEG test. Distribution of PEG in ascites might have caused a lower urinary excretion rate and thus underestimated possible permeability changes in patients with ascites. However, Kalaitzakis et al assessing intestinal permeability with 51Cr-EDTA concluded that a loss of 51Cr-EDTA into the ascitic compartment was unlikely and paracentesis had no significant effect on the urinary 51Cr-EDTA excretion, which suggests that ascites in itself does not unduly affect the test results[5]. In the present study, ascitic fluid from three cirrhotic patients was tested for PEG and it was not detected; therefore, the possibility of lower urinary excretion rates due to distribution of PEG in ascites can be ruled out.

Previous studies have shown an association between IPI and severity of LC assessed according to the Child-Pugh classification[8,9], but other studies have failed to reproduce these results[29]. In the present study, we observed significantly higher PEG 3350 retrieval and IPI in Child-Pugh class C patients as compared with that in class A and B patients. Methodological and/or patient selection differences should be taken into account when
interpolating the results of this study.

In this study, there were no concomitant infections and a significantly higher TNF-α level in cirrhotic patients with ascites than healthy control subjects or patients with LC with ascites was seen; thus, there was a tendency for a negative correlation between the TNF-α level and Child-Pugh class in the advanced stage of LC. In advanced cirrhosis, hepatic damage and inflammation are reduced due to a decreased liver reserve and marked fibrosis, and consequently, ALT levels decrease. Additionally, diminished amounts of cytokine-producing cells such as hepatocytes and Kupffer cells may lead to a decrease of TNF-α production[31]. In LC, several inflammatory states and commonly occurring infections may be another source of TNF-α production and could explain contradicting results. Increased production of TNF-α associated with inflammation and tissue necrosis is seen not only in hepatitis, but also in other inflammatory conditions. In the present study, IPI and urinary nitrite excretion were significantly higher in patients with LC with ascites as compared to patients with LC without ascites or healthy control subjects, with a significant correlation. Since NO is thought to have a wide range of biological functions other than vasodilatation, it is likely to affect both the progress and the clinical features of LC as well as the hemodynamics in cirrhotic patients. For example, NO is a potent inducer of increased membrane permeability in the vascular endothelium and intestinal mucosa, possibly contributing to the accumulation of ascites and to bacterial translocation[31]. In the current study, although TNF-α was thought to induce NO synthesis (NOS) through the inducible NOS and endothelial NOS[32,33], there was no significant correlation between TNF-α level and NO level in LC. This is the same to some studies, where such a relation could not be observed[34,35]. It has been suggested that some other factors including TNF-α contribute to elevation of NO in LC.

A simple comparison of the published data with the findings of the present study is not easy to make. There were differences between the reported results of intestinal permeability, which means differences in the methods of assessment, including the composition of the probe solution and analytic techniques employed, as well as differences in the patient populations and in the causes and severity of LC. In conclusion, our results suggest that increased intestinal macromolecular permeability and NO are probably of importance in the pathophysiology and progression of LC with ascites, and furthermore, IPI may be a contributory factor in the development of encephalopathy in LC.

ACKNOWLEDGMENTS

We express our gratitude to Ho Young Na and Gun Young Hong for assisting with the sample collection and Cheol Hyun Kim for assistance with the statistical analysis.

REFERENCES

1. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology 1995; 108: 1566-1581
2. DeMeo MT, Mutlu EA, Keshavarzian A, Tebin MC. Intestinal permeation and gastrointestinal disease. J Clin Gastroenterol 2002; 34: 385-396
3. Budillon G, Porrilli G, Pacella M, Cuomo R, Menzies IS, Investition of intestine and liver function in cirrhosis using combined sugar oral loads. J Hepatol 1985; 1: 515-524
4. Farhadi A, Banan A, Fiedls J, Keshavarzian A. Intestinal barrier: an interface between health and disease. J Gastroenterol Hepatol 2003; 18: 479-497
5. Kalaitzakis E, Johansson JE, Bjarnason I, Bjorsson E. Intestinal permeability in cirrhotic patients with and without ascites. Scand J Gastroenterol 2006; 41: 326-330
6. Fujii T, Seki T, Maruoka M, Tanaka J, Kawashima Y, Watanabe T, Sawamura T, Inoue K. Lactulose-L-rhamnose intestinal permeability test in patients with liver cirrhosis. Hepatol Res 2001; 19: 158-169
7. Huglo D, De Botton S, Canva-Delcambre V, Colombel JF, Wallaert B, Steinling M, Marchandise X. Simultaneous determination of pulmonary and intestinal permeability in patients with alcoholic liver cirrhosis. Eur J Nucl Med 2001; 28: 1505-1511
8. Campillo B, Pernet P, Bories PN, Richardet JP, Devanlay M, Ausset C. Intestinal permeability in liver cirrhosis: relationship with severe septic complications. Eur J Gastroenterol Hepatol 1999; 11: 755-759
9. Pascual S, Such J, Esteban A, Zapater P, Casellas JA, Aparicio JR, Girona E, Gutierrez A, Carnices F, Palazon JM, Sola- Vera J, Perez-Mateo M. Intestinal permeability is increased in patients with advanced cirrhosis. Hepatogastroenterology 2003; 50: 1482-1486
10. Zuckerman MJ, Menzies IS, Ho H, Gregory GG, Casner NA, Crane RS, Hernandez JA. Assessment of intestinal permeability and absorption in cirrhotic patients with ascites using combined sugar probes. Dig Dis Sci 2004; 49.
11 Michele HR, Manogue Kr, Spriggs DR, Revhaug A, ODwyer S, Dinarello CA, Cerami A, Wolff SM, Wilmore DW. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988; 318: 1481-1486

12 ODwyer ST, Michele HR, Ziegler TR, Revhaug A, Smith RJ, Wilmore DW. A single dose of endotoxin increases intestinal permeability in healthy humans. Arch Surg 1988; 123: 1459-1464

13 Odeh M, Sabo E, Srugo I, Oliven A. Serum levels of tumor necrosis factor-alpha correlate with severity of hepatic encephalopathy due to chronic liver failure. Liver Int 2004; 24: 110-116

14 Kiki I, Yilmaz O, Erdem F, Gundogdu M, Demircan B, Bilici M. Tumor necrosis factor-alpha levels in hepatitis B virus-related chronic active hepatitis and liver cirrhosis and its relationship to Knodell and Child-Pugh scores. Int J Clin Pract 2006; 60: 1075-1079

15 Zang W, Yue B, Wang GQ, Lu SL. Serum and ascites levels of macrophage migration inhibitory factor, TNF-alpha and IL-6 in patients with chronic viral hepatitis B and hepatitis cirrhosis. Hepatobiliary Pancreat Dis Int 2002; 1: 577-580

16 Giron-Gonzalez JA, Martinez-Sierra C, Rodriguez-Ramos C, Macias MA, Rendon P, Diaz F, Fernandez-Gutierrez M, Martin-Herrera L. Implication of inflammation-related cytokines in the natural history of liver cirrhosis. Liver Int 2004; 24: 437-445

17 Lee FY, Lu RH, Tsai YT, Lin HC, Hou MC, Li CP, Liao TM, Lin LF, Wang SS, Lee SD. Plasma interleukin-6 levels in patients with cirrhosis. Relationship to endotoxemia, tumor necrosis factor-alpha, and hyperdynamic circulation. Scand J Gastroenterol 1996; 31: 500-505

18 Eriksson AS, Greter C, Wallerstedt S. Elevation of cytokines in peritoneal fluid and blood in patients with liver cirrhosis. Hepatogastroenterology 2004; 51: 505-509

19 Vallance P, Moncada S. Hyperdynamic circulation in cirrhosis: a role for nitric oxide? Lancet 1991; 337: 776-778

20 Oudenhoven IM, Klaassen HL, Lapre JA, Weerkamp AH, Van der Meer R. Nitric oxide-derived urinary nitrate as a marker of intestinal bacterial translocation in rats. Gastroenterology 1994; 107: 47-53

21 DeMeo MT, Mutlu EA, Keshavarzian A, Tobin MC. Intestinal permeation and gastrointestinal disease. J Clin Gastroenterol 2002; 34: 385-396

22 Rahman SH, Ammori BJ, Larvin M, McMahon MJ. Increased nitric oxide excretion in patients with severe acute pancreatitis: evidence of an endotoxin mediated inflammatory response? Gut 2003; 52: 270-274

23 Keshavarzian A, Holmes EW, Patel M, Iber F, Fields JZ, Pethkar S. Leaky gut in alcoholic cirrhosis: a possible mechanism for alcohol-induced liver damage. Am J Gastroenterol 1999; 94: 200-207

24 McKay DM, Baird AW. Cytokine regulation of epithelial permeability and ion transport. Gut 1999; 44: 283-289

25 Wallace JI, Miller MJ. Nitric oxide in mucosal defense: a little goes a long way. Gastroenterology 2000; 119: 512-520

26 Banan A, Fields JZ, Zhang Y, Keshavarzian A. iNOS upregulation mediates oxidant-induced disruption of F-actin and barrier of intestinal monolayers. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1234-G1246

27 Philipson EK, Batsberg W, Christensen AB. Gastrointestinal permeability to polyethylene glycol: an evaluation of urinary recovery of an oral load of polyethylene glycol as a parameter of intestinal permeability in man. Eur J Clin Invest 1988; 18: 139-145

28 Parlesak A, Bode JC. Parallel determination of gut permeability in man with M(r) 400, M(r) 1500, M(r) 4000 and M(r) 10,000 polyethylene glycol. Eur J Clin Chem Clin Biochem 1994; 32: 813-820

29 Parlesak A, Schafer C, Schutz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol 2000; 32: 742-747

30 Loret S, Nollevaux G, Remacle R, Klimke M, Barakat I, Deloyer P, Grandfils C, Dandriofosse G. Analysis of PEG 400 and 4000 in urine for gut permeability assessment using solid phase extraction and gel permeation chromatography with refractometric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 805: 195-202

31 Guarner C, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, Vilardell F, Mourreelle M, Moncada S. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. Hepatology 1993; 18: 1139-1143

32 Elsing C, Harenberg S, Stremler W, Herrmann T. Serum levels of soluble Fas, nitric oxide and cytokines in acute decompensated cirrhotic patients. World J Gastroenterol 2007; 13: 421-425

33 Genesca J, Gonzalez A, Segura R, Catalan R, Marti R, Varela E, Cadelina G, Martinez M, Lopez-Talavera JC, Esteban R, Groszmann RJ, Guardia J. Interleukin-6, nitric oxide, and the clinical and hemodynamic alterations of patients with liver cirrhosis. Am J Gastroenterol 1999; 94: 169-177

34 Barsacchi R, Ferrotta C, Bulotta S, Moncada S, Borgese N, Clementi E. Activation of endothelial nitric-oxide synthase by tumor necrosis factor-alpha: a novel pathway involving sequential activation of neutral sphingomyelinase, phosphatidylinositol-3' kinase, and Akt. Mol Pharmacol 2003; 63: 886-895

35 Wiest R, Das S, Cadelina G, Garcia-Tsao G, Milstien S, Groszmann RJ. Bacterial translocation in cirrhotic rats stimulates eNOS-derived NO production and impairs mesenteric vascular contractility. J Clin Invest 1999; 104: 1223-1233

S- Editor Li DL  L- Editor Rippe RA  E- Editor Zhang WB