

# Changes of plasma D(-)-lactate, diamine oxidase and endotoxin in patients with liver cirrhosis

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**BACKGROUND:** Plasma D(-)-lactate and diamine oxidase (DAO) can reflect patients' intestinal mucosal condition. We evaluated the changes of plasma D(-)-lactate, DAO and endotoxin activities and their significance in patients with liver cirrhosis.

**METHODS:** Fifty liver cirrhosis patients were enrolled into experimental group and 30 healthy people into control group. The plasma levels of D(-)-lactate, DAO and endotoxin were detected spectrophotographically.

**RESULTS:** The level of D(-)-lactate was significantly higher in the experimental group than that in the control group ( $P < 0.01$ ). Significant differences of D(-)-lactate levels were observed in Child-Pugh subgroups of the experimental group ( $P < 0.01$ ). The level of DAO was significantly higher in the experimental group than that in the control group ( $P < 0.01$ ), but the level of DAO in Child-Pugh subgroup C was significantly lower than that in Child-Pugh subgroup B ( $P < 0.01$ ). The level of endotoxin was significantly increased in the experimental group except Child-Pugh subgroup A ( $P < 0.01$ ). The plasma levels of D(-)-lactate, DAO and endotoxin were positively correlated with each other ( $P < 0.01$ ).

**CONCLUSIONS:** The data suggest that both plasma D(-)-lactate and DAO activity are sensitive markers for early diagnosis of gut failure and endotoxemia in patients with liver cirrhosis. The impairment of intestinal barrier function may be one of the critical reasons for deterioration of liver cirrhosis.

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**KEY WORDS:** liver cirrhosis; D(-)-lactate; diamine oxidase; endotoxin; intestinal permeability; gut failure

## Introduction

Increased gut permeability and endotoxemia play a critical role in multiple organ failure.<sup>[1]</sup> Endotoxemia and small intestinal bacterial overgrowth often occur in patients with liver disease.<sup>[2]</sup> Since endotoxemia, intestinal bacterial overgrowth and increased gut permeability can form a vicious circle,<sup>[3]</sup> it is very important to evaluate intestinal permeability and mucosal impairment of patients with liver disease. Evaluation of intestinal permeability include gas chromatography of ethylene diamine tetraacetic acid<sup>[4]</sup> or sugar molecules.<sup>[5]</sup> Unfortunately, these tests are poorly used clinically because of their complicated procedures.

Endotoxemia and intestinal barrier failure deteriorate liver cirrhosis. D(-)-lactate is produced by bacteria in the human gastrointestinal tract. In mammal tissues, there is no enzyme system for metabolism of D(-)-lactate. Evaluation of plasma D(-)-lactate indicates promptly the change of intestinal permeability.<sup>[6]</sup> Since diamine oxidase (DAO) in layer of villi of the intestinal mucosa reflects the function and structure of the small intestine, the plasma level of DAO can be used as a marker for evaluation of maturation and integrity of the intestinal mucosa.<sup>[7]</sup> Moreover, endotoxin may greatly aggravate injury of the intestinal mucosa in patients with liver cirrhosis. In this study we measured the plasma levels of D(-)-lactate, DAO and endotoxin and their changes in 50 patients with liver cirrhosis.

## Methods

### Patients

Fifty patients with liver cirrhosis, who had been treated at the Department of Infectious Diseases, Renmin Hospital, Wuhan University from October 2002 to March 2003, were studied, and 30 healthy volunteers served as controls. The inclusion criteria for the patients were consistent with the Chinese Protocol for Prophylaxis and Treatment of Viral Hepatitis issued by the Chinese Society for Infectious Diseases and Parasitic Diseases.<sup>[8]</sup> Child-Pugh classification was used to evaluate liver func-

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tion.<sup>[9]</sup> The causes of liver cirrhosis in all patients were infection of hepatitis B virus. The patients consisted of 36 men and 14 women, aged from 18 to 61 years ( $41.7 \pm 11.7$ ), and none of them had gastrointestinal infection, ulceration, or renal diseases. The diagnosis of liver cirrhosis was based on clinical history, symptoms, and signs, hematological data, and ultrasonographic or CT findings. There were no statistical differences in age and gender between the patients and controls.

### Reagents and methods

Plasma was collected for determination of D(-)-lactate, DAO and endotoxin. The level of D(-)-lactate was measured using the enzymatic-spectrophotometric method.<sup>[6]</sup> D(-)-lactate standard stock solution and D-lactic dehydrogenase were purchased from Sigma Chemical Company, USA. DAO was detected using the o-diamisidine reagent method.<sup>[10]</sup> O-diamisidine, cadaverine dihydrochloride, horseradish peroxidase, and DAO standard stock solution were also purchased from Sigma Chemical Company, USA. The level of endotoxin was determined using a chromogenic limulus amoebocyte lysate test kit according to the manufacturer's instructions (Shanghai Biochemical Institute, Shanghai, China).<sup>[11]</sup>

### Statistical analysis

The data were expressed as mean  $\pm$  SD. Student's *t* test, ANOVA and linear correlation analysis using SPSS software (11.5 edition) were used.

### Results

The plasma levels of D(-)-lactate, DAO and endotoxin were significantly higher in the patients than in the controls ( $P < 0.01$ , Table 1).

The plasma levels of D(-)-lactate, DAO and endotoxin in different Child-Pugh subgroups are shown in Table 2. The levels of D(-)-lactate and DAO of the patients were significantly higher than those of the controls ( $P < 0.01$ ). Endotoxin levels of Child-Pugh subgroups B and C were also significantly higher than those of the controls ( $P < 0.01$ ); but there was no significant difference in endotoxin level between Child-Pugh subgroup A and the controls ( $P > 0.05$ ). The comparison of different Child-Pugh subgroups showed that the DAO le-

**Table 1.** The results of plasma D(-)-lactate, DAO and endotoxin in patients with liver cirrhosis (mean  $\pm$  SD)

Groups	<i>n</i>	D(-)-lactate (mg/L)	DAO (U/mL)	Endotoxin (U/mL)
Subjects	50	$13.21 \pm 9.15^*$	$5.07 \pm 1.80^*$	$0.72 \pm 0.25^*$
Controls	30	$0.77 \pm 0.25$	$1.93 \pm 0.75$	$0.48 \pm 0.19$

\* :  $P < 0.01$ .

**Table 2.** The alterations of plasma D(-)-lactate, DAO and endotoxin in different Child-Pugh groups (mean  $\pm$  SD)

Groups	<i>n</i>	D(-)-lactate (mg/L)	DAO (U/mL)	Endotoxin (U/mL)
Child-Pugh A	19	$6.56 \pm 2.26^*$	$4.07 \pm 1.27^*$	$0.58 \pm 0.12^*$
Child-Pugh B	17	$11.60 \pm 5.44^*$	$6.79 \pm 1.85^*$	$0.75 \pm 0.17^*$
Child-Pugh C	14	$23.77 \pm 8.99^*$	$4.35 \pm 0.91^*$	$1.06 \pm 0.20^*$
Controls	30	$0.77 \pm 0.25$	$1.93 \pm 0.75$	$0.48 \pm 0.19$

\* :  $P < 0.01$ .

**Table 3.** Correlation of the levels of D(-)-lactate, DAO and endotoxin

y/x	<i>r</i>	<i>P</i> <
DAO/endotoxin	0.321	0.01
D(-)-lactate/DAO	0.413	0.01
D(-)-lactate/endotoxin	0.658	0.01

vel of Child-Pugh subgroup B was significantly higher than that of Child-Pugh subgroup C ( $P < 0.01$ ); but there was no significant difference between Child-Pugh subgroups C and A ( $P > 0.05$ ). The linear correlation analysis of plasma levels of D(-)-lactate, DAO, endotoxin showed that the plasma level of DAO was positively correlated with that of endotoxin in the patients ( $r = 0.321$ ,  $P < 0.01$ , Table 3). This positive relationship was also found between the plasma levels of DAO and D(-)-lactate ( $r = 0.413$ ,  $P < 0.01$ ) as well as between levels of endotoxin and D(-)-lactate ( $r = 0.658$ ,  $P < 0.01$ ).

### Discussion

D(-)-lactate, a product of bacterial fermentation, is produced by many bacteria in the gastrointestinal tract. Not produced and not metabolized in mammal tissues, or even it is found in these tissues, its metabolism is very slow. In general, the plasma level of D(-)-lactate in humans is quite low.<sup>[12]</sup> During gut failure, intestinal permeability is increased, leading to an efflux of bacteria and their metabolic products into the circulation, including D(-)-lactate. Therefore, D(-)-lactate is a useful early predictor for elevation of intestinal permeability and gut failure. Munray et al<sup>[13]</sup> demonstrated that plasma levels of D(-)-lactate in patients with mesenteric ischemia were significantly elevated compared with those of patients with acute or insidious abdominal diseases. D(-)-lactate level in blood was considered a useful marker for the diagnosis of gut failure caused by acute mesenteric ischemia.<sup>[13]</sup>

DAO, a high-activity intracellular enzyme, catalyzes and metabolizes histamine, putrescine and cadaverine and presents predominantly within the intestinal mucosa,

kidney and placenta but little in plasma.<sup>[14]</sup> DAO makes putrescine oxidized into amino butylaldehyde and cyclized into pyrrole. The activity of DAO is closely associated with villi height, nucleic acid, and protein synthesis of intestinal mucosal cells.<sup>[15]</sup> When the small intestinal barrier is injured, intestinal mucosal cells exfoliate into the bowel lumen, making the activity of mucosal DAO decreased. When DAO enters the lymphatic vessel in the intercellular space and blood stream, the plasma level of DAO is increased. Therefore, the high activities of plasma DAO reflect the impairment degree of the intestinal tract. Many investigators suggested that combined determination of plasma DAO activity and the ratio of urine lactulose to mannitol excretion could judge the structural and functional alterations of the intestinal mucosa. The activity of plasma DAO reflects intestinal permeability more validly and promptly.<sup>[16]</sup>

In this study we determined alterations of plasma D(-)-lactate, DAO and endotoxin in order to evaluate the function of intestinal barrier and permeability in patients with liver cirrhosis. The level of endotoxin was normal, but the plasma levels of D(-)-lactate, and DAO were increased in Child-Pugh subgroup A. The results indicated that impairment of intestinal barrier and high intestinal permeability exist even in patients with early stage liver cirrhosis without endotoxemia. In Child-Pugh subgroup B, the levels of the three markers increased markedly, but in Child-Pugh subgroup C, plasma levels of D(-)-lactate and endotoxin peaked, while DAO decreased. The changes of D(-)-lactate and DAO suggested serious injury of the intestinal barrier, high intestinal permeability, and endotoxemia with deterioration of liver function. Hence, intestinal barrier dysfunction and high intestinal permeability play an important role in the pathogenesis of bacterial translocation, infection and endotoxemia in patients with liver cirrhosis,<sup>[17]</sup> which aggravate liver failure and multiple organ failure. The defense mechanisms of the normal intestinal tract include ecological equilibrium of the intestinal lumen flora,<sup>[18]</sup> mechanical factors of the intestinal lumen mucosal barrier,<sup>[19]</sup> an intact immune response in the intestinal tract,<sup>[20]</sup> and the gut-liver feedback axis. These factors maintain the physiological condition of the intestine to avoid bacterial overgrowth and prevent endotoxemia.<sup>[21]</sup> In patients with liver cirrhosis, many factors such as the disappearance of migrating motor complex (MMC) of the small intestine,<sup>[22]</sup> the changes of gut hormones and inflammation media and neurotransmitters,<sup>[23-25]</sup> and gut stasis can lead to dysfunction and failure of the intestinal barrier and overgrowth of bacteria in the small intestine (especially G-flora).<sup>[26,27]</sup>

Excessive proliferation of the intestinal flora will impair mesenteric cell architecture and shorten or exfoliate the intestinal villi.<sup>[28]</sup> Endotoxins produced by G-bacteria pass through the intestinal mucosa into blood and impair the liver directly by activating Kupffer cells

which produce inflammatory mediators, with hypoxia and acidosis of the intestinal mucosa, increased intestinal permeability, and bacterial translocation.<sup>[29,30]</sup> In this study Child-Pugh subgroup C showed not only increased intestinal permeability, but also bacterial overgrowth of the small intestine,<sup>[31]</sup> which contributed to the peak level of D(-)-lactate. As to DAO, mucosal lesions of the intestine lead to temporary elevation of the plasma level of DAO because of impairment of the intestinal epithelium and DAO pouring into blood. In advanced stage of gut failure due to serious necrosis and exfoliation of intestinal mucosal cells, the plasma level of DAO was decreased.<sup>[7]</sup> In this study, the low plasma level of DAO in Child-Pugh subgroup C was also due to serious gut failure and endotoxemia. It was reported that in patients with liver cirrhosis, the plasma level of endotoxin was elevated and the increasing rate was dependent on the impairment severity of liver function.<sup>[32,33]</sup> Our results have confirmed this finding. The positive relationship between levels of D(-)-lactate and endotoxin in this study indicated that D(-)-lactate might be a specific predictor for endotoxemia, and that both plasma D(-)-lactate and DAO levels are sensitive markers in early diagnosis of gut failure. Bacterial overgrowth of the small intestine, increased intestinal permeability, and endotoxemia play an important role in deterioration of liver cirrhosis.

## Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

## References

- 1 Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. *Gastroenterology* 1995;108:1566-1581.
- 2 Xie GQ, Jiang TX, Chen YH, Liu DW, Zhu DF, Wang ZG. Induction of acute hepatic injury by endotoxin in mice. *Hepatobiliary Pancreat Dis Int* 2002;1:558-564.
- 3 Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2001;48:206-211.
- 4 Travis S, Menzies I. Intestinal permeability: functional assessment and significance. *Clin Sci* 1992;82:471-488.
- 5 Fujii T, Seki T, Maruoka M, Tanaka J, Kawashima Y, Watanabe T, et al. Lactulose-L-rhamnose intestinal permeability test in patients with liver cirrhosis. *Hepato Res* 2001;19:158-169.
- 6 Murray MJ, Barbose J, Cobb CF. Serum D(-)-lactate levels as a predictor of acute intestinal ischemia in a rat model. *J Surg Res* 1993;54:507-509.
- 7 Wu CT, Li ZL. Effect of DAO on intestinal damage in acute necrotizing pancreatitis in dogs. *World Chin J Digestol* 1999;7:

- 64-65.
- 8 Chinese Society for Infectious Diseases and Parasitic Diseases. Protocol for Prophylaxis and Treatment of Viral Hepatitis. *Chin J Hepatol* 2000;8:324-330.
  - 9 Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646-649.
  - 10 Hosoda NM, Nishi MN, Nakagawa M, Hiramatsu Y, Hioki K, Yamamoto M. Structural and functional alterations in the gut of parenterally or enterally fed rats. *J Surg Res* 1989;47:129-133.
  - 11 Yao YM, Tian HM, Wang YP, Yu Y, Shi ZG, Sheng ZY. Introduction of a new limulus test: using PCA to disposal plasma to assay endotoxin level. *Shanghai J Med Lab* 1993;8:31-33.
  - 12 Marcos MA, Vila J, Gratacos J, Brancos MA, Jimenez de Anta MT. Determination of D-lactate concentration for rapid diagnosis of bacterial infections of body fluids. *Eur J Clin Microbiol Infect Dis* 1991;10:966-969.
  - 13 Murray MJ, Gonze MD, Nowak LR, Cobb CF. Serum D(-)-lactate levels as an aid to diagnose acute intestinal ischemia. *Am J Surg* 1994;167:575-578.
  - 14 Takagi K, Nakao M, Ogura Y, Nabeshima T, Kunii A. Sensitive colorimetric assay of serum diamine oxidase. *Clin Chim Acta* 1994;226:67-75.
  - 15 T Bieganski, J Kusche. Distribution and properties of human intestinal diamine oxidase (DAO) and its relevance catabolism. *BBA* 1993;756:196-203.
  - 16 Zhao H, Li XO, Lou GQ, Wang P, Cheng WP, Tu SX, et al. Research on intestine permeability in patients with post-hepatitis cirrhosis. *Chin J Infect Dis* 2002;20:105-107.
  - 17 Xu WH, Wu X J, Li JS. Influence of portal pressure change on intestinal permeability in patients with portal hypertension. *Hepatobiliary Pancreat Dis Int* 2002;1:510-514.
  - 18 Livein V, Peiffer I, Hudault S, Rochat F, Brassart D, Neeser JR, et al. Bifidobacterium strains from resident infant human gastrointestinal microflora exert antimicrobial activity. *Gut* 2000;47:646-652.
  - 19 Porter EM, van Dam E, Valore EV, Ganz T. Broad-spectrum antimicrobial activity of human intestinal defensin 5. *Infect Immun* 1997;65:2396-2401.
  - 20 Kagoff MF. Mucosa immunology: new frontiers. *Immunology Today* 1997;17:57-59.
  - 21 Swank GM, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. *World J Surg* 1996;20:411-417.
  - 22 Bouhnik Y, Alain S, Attar A, Flourie B, Raskine L, Sanson-Le Pors MJ, et al. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am J Gastroenterol* 1999;94:1327-1331.
  - 23 Alican I, Kubes P. A critical role for nitric oxide in intestinal barrier function and dysfunction. *Am J Physiol* 1996;270:G225-237.
  - 24 Yao YM, Bahrami S, Redl H, Schlag G. Monoclonal antibody to tumor necrosis factor- $\alpha$  attenuates hemodynamic dysfunction secondary to intestinal ischemia reperfusion in rats. *Crit Care Med* 1996;24:1547-1553.
  - 25 Menconi MJ, Salzman AL, Unno N, Ezzell RM, Casey DM, Brown DA, et al. Acidosis induces hyperpermeability in Caco-2BBE cultured intestinal epithelial monolayers. *Am J Physiol* 1997;272:G1007-1021.
  - 26 Qin HL, Su ZD, Gao Q, Lin QT. Early intrajejunal nutrition: bacterial translocation and gut barrier function of severe acute pancreatitis in dogs. *Hepatobiliary Pancreat Dis Int* 2002;1:150-154.
  - 27 Kirsch M. Bacterial overgrowth. *Am J Gastroenterol* 1990;85:231-237.
  - 28 Aldersley MA, Howdle PD. Intestinal permeability and liver disease. *Eur J Gastroenterol Hepatol* 1999;11:401-403.
  - 29 Zhao LF, Li H, Han DW. Intestinal endotoxemia and liver diseases. *World Chin J Digestol* 2000;8:1145-1149.
  - 30 Li XH, Gong JP, Shi YJ, Liu CA, Peng Y. In vitro expression of CD14 protein and its gene in kupffer cells induced by lipopolysaccharide. *Hepatobiliary Pancreat Dis Int* 2003;2:571-575.
  - 31 Morencos FC, de las Heras Castano G, Martin Ramos L, Lopez Arias MJ, Ledesma F, Pons Romero F. Small bowel bacterial overgrowth in patients with alcoholic cirrhosis. *Dig Dis Sci* 1995;4:1252-1256.
  - 32 Lin RS, Lee FY, Lee SD, Tsai YT, Lin HC, Lu RH, et al. Endotoxemia in patients with chronic liver disease: relationship to severity of liver disease, presence of esophageal varices, and hyperdynamic circulation. *J Hepatol* 1995;22:165-172.
  - 33 Yang YJ, Shi JS, Xie SM, Zhang DT, Cui BS. Effects of different drainage procedures on levels of serum endotoxin and tumor necrosis factor in patients with malignant obstructive jaundice. *Hepatobiliary Pancreat Dis Int* 2003;2:426-430.

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