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ELEVATED PLASMA LEVELS OF NATURAL ANTIMICROBIAL PEPTIDES IN PATIENTS WITH CHRONIC LIVER DISEASE

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BACKGROUND

The pathogenesis of infection in patients with end stage liver disease remains poorly understood (*Bonnel et al. Clin Gastroenterol Hepatol 2011*). Translocation of gut bacteria may play a pivotal role. Defensins are natural antimicrobial peptides with activity against a wide range of microorganisms. In humans, α-defensins are secreted by PMNs (HNP-1 to -4) or Paneth cells (HD-5 and HD-6), whereas β-defensins are broadly expressed (*Ganz. Nature Rev Immunol, 2003*). Localization of α- and β-defensins to the small and large intestinal epithelium indicates a role of these proteins in the defense against systemic translocation of commensal bacteria (*Wehkamp et al. Nature Clin Pract Gastroenterol Hepatol, 2005*). The potential involvement of deficiencies in the system of natural antimicrobial peptides (circulating and intestinal) in increased susceptibility of cirrhotic patients to infection has not been explored yet.

AIM OF THE STUDY

The aim of the present study was to determine the serum protein content for human defensin β1 (hBD-1) and β2 (hBD-2) in patients with chronic liver disease and associate their expression to markers of systemic bacterial translocation.

PATIENTS AND METHODS

Plasma was obtained from 24 patients with chronic viral hepatitis, 22 with decompensated cirrhosis, and 13 healthy controls. Demographic, clinical, and laboratory information is shown in Table 1.
Enzyme-linked Imunosorbent assays (ELISA) were used for the measurement of plasma concentrations of hBD-1 and hBD-2 (antibodies from Peprotech, UK) and lipopolysaccharide-binding protein/LBP (commercial kit from USCN, China).
Statistical analysis was performed with the GraphPad statistical software.

Table 1. Characteristics of the patient population

	Hepatitis	*Cirrhosis*
Patients (N)	24	22
Age (years, average ±SD,range)	43.5 ± 13 (26-65)	62.1 ± 11.7 (51-89)
Male	18	19
HBV -related	12	3
HCV - related	12	5
Alcohol - related		12
Unknown etiology		2
TBil (average±SD,range)	0.7 ± 0.42 (0.2-1.7)	3.57 ± 3.9 (0.32-15.73)
Crea (average±SD,range)	0.85 ± 0.15 (0.6-1.1)	1.04 ± 0.47 (0.6-2.5)
Meld score (average±SD,range)		15.9 ± 5.7 (8-25)

SUMMARY & CONCLUSIONS

- Plasma levels of hBD-1 and hBD-2 are significantly elevated in patients with chronic viral hepatitis and cirrhosis, independent of the underlying cause of liver disease.
- Both hBD-1 and hBD-2 are detected in the ascitic fluid of patients with cirrhosis.
- Elevated circulating β-defensins may be indicative of increased bacterial translocation as shown by the positive association between serum hBD2 and LBP.

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The authors report no conflict of interest.

RESULTS

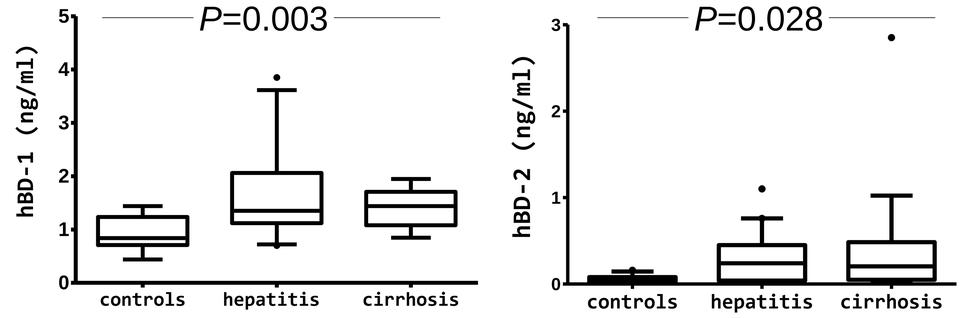

Figure 1. Plasma concentrations of hBD-1 and hBD-2 are significantly elevated in patients with viral hepatitis and cirrhosis. hBD-1 and hBD-2 concentrations in plasma samples were measured by ELISA. hBD-1 levels were higher in patients with chronic viral hepatitis (N=23, median, 95%CI, 1.35, 1.26-1.87) or cirrhosis (N= 15, 1.44, 1.2-1.57) than in controls (N=13, 0.84, 0.75-1.138) (P=0.003, Kruskal-Wallis test). Similarly, hBD-2 concentration was significantly increased in chronic viral hepatitis (N= 19, 0.24, 0.14-0.43) or cirrhosis (N=18, 0.21, 0.08-0.74) compared with healthy controls (N=11, 0.04, 0.03-0.08) (P=0.028).

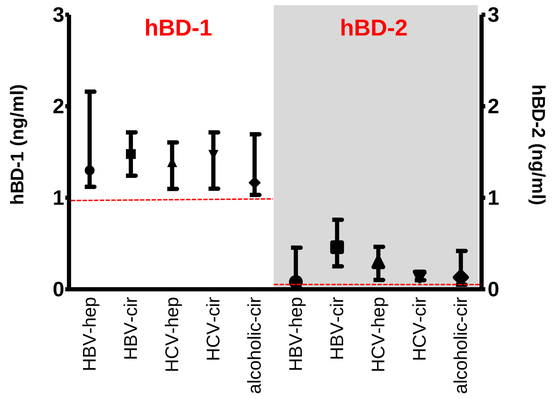

Figure 2. Etiology of chronic liver disease does not affect plasma concentration of hBD-1 and hBD-2. Plasma concentration of hBD-1 and hBD-2 was measured by ELISA in various subgroups of patients with chronic liver disease. The red dotted lines indicate the mean plasma concentration of hBD-1 and hBD-2 in the respective healthy controls.

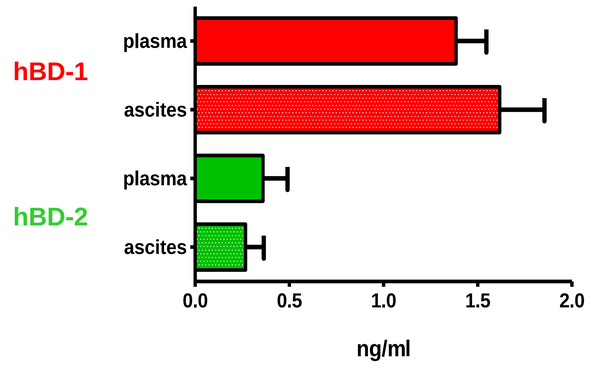

Figure 3. hBD-1 and hBD-2 proteins are detected in ascitic fluid of patients with cirrhosis. Ascites was obtained after peritoneal puncture of patients (n=5) with cirrhosis and hBD-1 and hBD-2 concentrations were detected by ELISA. Plasma samples of the same individuals were concomitantly obtained and tested for hBD-1 and hBD-2. Bars represent mean + sdv. (hBD-1: plasma: 1.39 ± 0.36 ng/ml, ascites: 1.62 ± 0.53 hBD-2: plasma: 0.36 ± 0.29, ascites:0.27 ± 0.22).

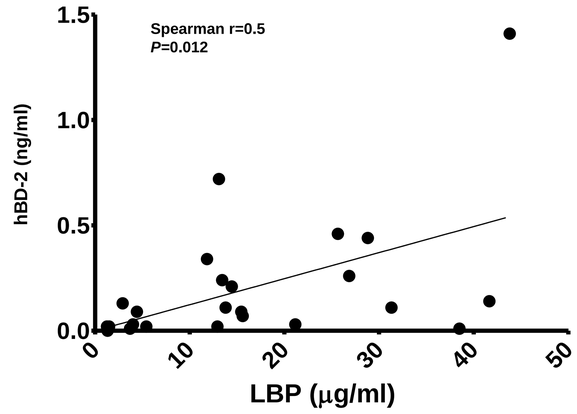

Figure 4. Positive correlation of LBP and hBD-2 in peripheral blood of cirrhotic patients. LBP and hBD-2 concentrations were measured in the sera of individual patients with cirrhosis. Correlation analysis revealed a significant association between values of the two markers.

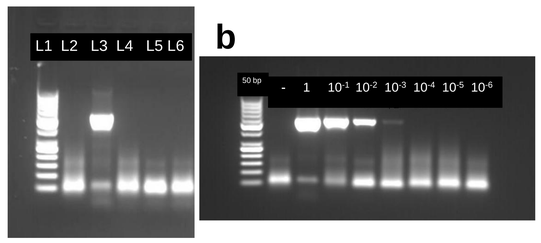

Figure 5. Bacterial DNA is not detected in the sera of cirrhotic patients, possibly due to a threshold limitation. **5a,** Total DNA was extracted from the serum of patients with cirrhosis and bacterial 16s ribosomal DNA (rRNA) was amplified by use of universal primers (F: 5'-AGAGTTTGATCATGGCTCAG-3' and R:5'-ACCGCGACTGCTGCTGGCAC-3'). L1, 50 bp DNA ladder, L2, negative control, L3, positive control, culture of S.aureus (540bp), L4-6, cirrhotic patients. **5b,** Total DNA was extracted from serial dilutions of cultured *S.aureus*. Results indicate a threshold (15.8 pg/ul) for the detection of bacterial DNA.