Diagnostic Microbiology and Infectious Disease xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

GP73, a new marker for diagnosing HBV-ACLF in population with chronic HBV infections ☆

Hongshan Wei [a,*,1], Jing Zhang [b,1], Hongmin Li [a], Hui Ren [a], Xiaohua Hao [a], Yubo Huang [a]

[a] Institute of Infectious Disease, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
[b] Beijing Youan Hospital, Capital Medical University, Beijing 100039, China

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form 9 January 2014
Accepted 10 January 2014
Available online xxxx

Keywords:
Golgi protein 73
Acute on chronic liver failure
HBV-associated acute-on-chronic liver failure
Chronic hepatitis B
Marker
Diagnosis

ABSTRACT

Although Golgi protein 73 (GP73) has been widely evaluated for diagnosing hepatocellular carcinoma (HCC) and other liver diseases in recent decade, its serum profile of patients with hepatitis B virus (HBV)–associated acute-on-chronic liver failure (HBV-ACLF) is still unknown. This study was designed to evaluate the serum levels of GP73 in patients with HBV-ACLF. The participants included 200 apparently healthy controls; 200 patients with chronic hepatitis B (CHB); 200 patients with HCC; 210 patients with HBV-ACLF, in which 29 HBV-ACLF patients were followed up for 3 months. All patients were Hepatitis B virus surface antigen (HBsAg) positive. The concentrations of GP73 in patients with HBV-ACLF (285.3 ± 128.5 ng/mL) were markedly higher than those HCC patients (159.1 ± 105.8 ng/mL), CHB patients (64.65 ± 44.99 ng/mL), and healthy controls (35.37 ± 12.41 ng/mL). When the cut-off value was set at 182.1 ng/mL, the sensitivity and specificity of HBV-ACLF diagnosis were 77.62% (95% confidence interval [CI]: 71.37%–83.07%) and 95.50% (95% CI: 92.27%–98.26%), respectively. If serum GP73 concentration was still above 361.6 ng/mL after 14 days of follow-up, the patient's prognosis may be depressed. Serum GP73 may be used to diagnosis HBV-ACLF in population with chronic HBV infections.

1. Introduction

The concept of acute-on-chronic liver failure (ACLF) was introduced recently to describe a subset of patients with chronic liver disease presenting with profound deterioration of liver function and rapidly evolving multi-organ failure (Garg et al., 2012a; Verbeke et al., 2011). ACLFs are characterized by simultaneous coexistence of 2 liver conditions. One of them is chronic or long-standing, and the other is acute or recent (Duseja et al., 2010). Without treatment with liver transplantation or molecular adsorbent recirculating system (MARS), only 17% patients can survived for 3 years (Hessel, 2006). Even if treated with the MARS, only 33% patients can survived for 3 years (Hessel et al., 2010). Early recognition of those with dismal prognosis may permit timely performing liver replacement or receiving supportive therapies (Radha Krishna et al., 2009).

Spontaneous reactivation of chronic hepatitis B (CHB) is an important cause of ACLF (Garg et al., 2011). In China, hepatitis B virus (HBV)–associated ACLF (HBV-ACLF) patients account for more than 80% of ACLF patients, owing to a high prevalence of chronic HBV infection (Zou et al., 2009). Since ACLF is a life-threatening event, early recognition and prognosis judgment are critical factors for the prevention and treatment of HBV-ACLF. The model for end-stage liver disease (MELD) scoring system was used to diagnose ACLF in patients with CHB (Sun et al., 2009). For patients with an MELD score of 30–40, a low viral load pretreatment and quick decline of HBV-DNA level are good predictors for the survival (Yu et al., 2008), but recent report demonstrated that the decreasing of HBV-DNA did not improve the short-term prognosis of patients with HBV-ACLF (Cui et al., 2010). Ascertaining a biomarker to evaluate the prognosis of patients with HBV-ACLF is still a challenge for clinicians.

At the beginning of this century, a novel 73-kDa human Golgi protein was found expression in hepatocytes (Kladney et al., 2000). It subsequently emerged that Golgi protein 73 (GP73) is a new marker for evaluating advanced liver diseases and hepatocellular carcinoma (HCC) (Kladney et al., 2002; Iftikhar et al., 2004). Then, several studies suggested that GP73 was over-expressed in a variety of acute and chronic liver diseases (Liu et al., 2011), and its serum concentration correlated with progression of chronic liver disease (Iftikhar et al., 2004; Sun et al., 2011). Recently, our results also demonstrated that serum GP73 levels were consistent with the grading of patients with chronic HBV infections (Wei et al., 2013), but its serum profile in patients with HBV-ACLF has not been elucidated. The present study was designed to evaluate the performance of GP73 for diagnosing patients with HBV-ACLF in population with chronic HBV infections.

☆ Declaration of competing interests. The authors declare that they have no competing interests.

* Corresponding author. Tel.: +86-10-84322620; fax: +86-10-84322148.
E-mail address: drwei@ccmu.edu.cn (H. Wei).

[1] Coauthor of this paper.

0732-8893/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.008

2. Materials and methods

2.1. Patients and design

All participants consecutively admitted to our medical centers (Beijing Ditan Hospital and Beijing Youan Hospital), between October 2010 and March 2012, including 200 apparently healthy controls, 200 CHB patients, 200 HCC patients, and 210 patients with HBV-ACLF. All patients were HBV positive. Each participant signed standard consent statement, before being recruited onto this study. The demographic data of all participants were described in Table 1. The study was approved by the Institutional Review Board of the Beijing Ditan Hospital, Capital Medical University.

The HBV-ACLF diagnosis was based on consensus recommendations of the Asian Pacific Association for The Study of the Liver (APASL) (Sarin et al., 2009). Briefly, the main points of inclusion criterion were: 1) HBsAg positive over 6 months, 2) prothrombin time (PT) ≤40%, and 3) total bilirubin (Tbil) ≥10 upper limit of normal. The CHB diagnosis was based on recommendations of the Institute of Medicine (Mitchell et al., 2010), and HCC diagnosis, based on recommendations of the National Cancer Institute Clinical Trials Planning Meeting (Thomas et al., 2010). The HCC diagnosis was confirmed by at least 2 imaging methods, such as computed tomography, magnetic resonance imaging, ultrasonic, and/or Alpha fetoprotein (AFP) ≥400 ng/mL. The patients with 1 or more exclusion criteria were eliminated from the cohort: 1) serious cardiac, lung, and kidney diseases; 2) co-infected chronically with Hepatitis C virus (HCV), HIV, and tuberculosis; 3) age below 18 years old; 4) HCC patients without chronic HBV infections were also excluded from HCC group; 5) the patients who received antiviral therapy at screening were also excluded from CHB and HCC groups. In 210 patients diagnosed with HBV-ACLF at baseline, 29 cases were followed up over 3 months (1, 2 weeks, 3 months), in which 9 patients died. Before initiating drug therapy, the serum samples were collected and stored at −70 °C.

2.2. Biochemical analysis

The liver function tests, including serum albumin, Tbil, and alanine aminotransferase (ALT), were measured using a Roche Hitachi 717 chemistry analyzer at the central laboratory of Beijing Ditan Hospital.

2.3. Quantitative determination of serum GP73

Quantitative determination of GP73 in serum was performed using commercially available enzyme-linked immunosorbent assay kits (Hotgen Biotech Inc., Beijing, China), according to the manufacturer's protocol.

2.4. Statistical analysis

Statistical analysis was performed using the GraphPad Prism 5.0.1. Student's *t* test was used to compare the difference of serum GP73 concentrations between different patients groups. Correlation between serum GP73 concentration and those of other biomarker was calculated using Pearson's correlation coefficient (*r*). Data were expressed as mean ± SEM or median. *P*-values <0.05 were considered to be statistically significant. The diagnostic performance of GP73 was evaluated by performing the area under the receiver operating characteristic (ROC) curve with 95% confidence interval (CI).

3. Results

3.1. Patient's characteristics

Table 1 summarized characteristics of control subjects and patients. The sex and age distribution among subclasses was shown. As the data shown in Table 1, no significant age differences were observed within any groups, although significant age differences were indicated between 4 groups ($F = 344.1$, $P < 0.0001$). The same trend was observed in serum GP73 concentrations in different groups and sex-related subgroups. These results suggested that sex and age are not related with GP73 difference between different groups.

3.2. Sensitivity and specificity of GP73 for diagnosing HBV-ACLF

Since HBV-ACLF is defined as a severe acute episode of CHB (Zhang et al., 2011), we selected CHB patients as control to perform ROC analysis. We firstly examined whether the concentration of GP73 significantly increased in HBV-ACLF patients. As expected, the concentrations of GP73 in patients with HBV-ACLF (285.3 ± 128.5 ng/mL) were markedly higher than those in HCC patients (159.1 ± 105.8 ng/mL), CHB patients (64.65 ± 44.99 ng/mL), or healthy controls (35.37 ± 12.41 ng/mL) (Fig. 1A). Results of ROC analysis showed that GP73 was an excellent marker for diagnosing HBV-ACLF. When the cut-off value was set at 182.1 ng/mL, the sensitivity and specificity of HBV-ACLF diagnosis were 77.62% (95% CI: 71.37%–83.07%) and 95.50% (95% CI: 92.27%–98.26%), respectively; when the cut-off value was set at 281.2 ng/mL, the sensitivity and specificity were 48.57% (41.67%–55.55%) and 99.22% (97.73%–99.84%), respectively (Fig. 1B). At this level (cut-off: 281.2 ng/mL), the sensitivity of GP73 diagnosed HCC in CHB population was 19.0% (Fig. 1C).

3.3. Dynamic monitoring GP73 is also a predictive marker for ACLF patients

To characterize the GP73 abnormalities for HBV-ACLF prognosis, 29 patients with HBV-ACLF were followed up over 3 months.

Table 1
Characteristics of all participants.

Category	Sex/ number	Mean (95% CI)				
		Age (y)	PT (s)	Tbil (μmol/L)	ALT (U/L)	ALB (g/L)
Control	M/126	37.55 (35.61–39.49)	11.49 (10.47–12.65)	11.26 (7.59–10.33)	24.68 (18.21–26.24)	46.35 (44.76–49.52)
	F/74	40.50 (38.04–42.96)				
CHB	M/138	34.97 (33.42–36.52)	12.86 (11.87–13.84)	21.49 (17.61–25.38)	92.09 (69.69–114.5)	44.92 (43.43–46.41)
	F/62	35.16 (33.02–37.30)				
HCC	M/162	54.21 (52.67–55.75)	14.67 (14.17–15.22)	51.42 (36.56–66.37)	54.47 (46.41–62.53)	39.05 (37.89–40.21)
	F/38	59.66 (56.79–62.52)[a]				
HBV-ACLF	M/139	49.31 (41.17–51.45)	30.52 (28.52–32.52)	337.6 (251.2–424.0)	147.2 (115.7–178.6)	29.86 (29.18–30.55)
	F/71	49.41 (45.40–53.42)				

[a] Compared with male HCC, $P = 0.002$.

Please cite this article as: Wei H, et al, GP73, a new marker for diagnosing HBV-ACLF in population with chronic HBV infections, Diagn Microbiol Infect Dis (2014), http://dx.doi.org/10.1016/j.diagmicrobio.2014.01.008