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Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research https://doi.org/10.1016/j.apjtm.2017.08.008

Elevated serum prostaglandin E2 predicts the risk of infection in hepatitis B virus-related acute-on-chronic liver failure patients

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ARTICLE INFO

Article history:
Received 14 Jun 2017
Received in revised form 12 Jul 2017
Accepted 18 Aug 2017
Available online 7 Oct 2017

Keywords:
Acute-on-chronic liver failure
Immune paralysis
Infection
Prostaglandin E2

ABSTRACT

Objective: To evaluate the serum Prostaglandin E2 (PGE2) level in Acute-on-chronic liver failure (ACLF) and determine its predicative value for infection.
Methods: From April 2014 to April 2015, ninety-one patients with hepatitis B virus and ACLF but without infection were enrolled into this prospective study that was carried out at our Hospital. Twenty patients with stable chronic hepatitis B were enrolled from the outpatient department and twenty healthy control subjects without any disease were enrolled from hospital staff. Serum PGE2 levels were determined using ELISA at enrollment. Clinical and laboratory parameters were collected. Receiver operating characteristic (ROC) curves were used to determine optimal cut-off values to predict infection.
Results: Significantly higher PGE2 levels were found in patients with ACLF in comparison with healthy controls and patients with stable CHB ($P < 0.0001$). In ACLF patients, PGE2 levels were significantly higher in patients that eventually developed infection than those without this complication ($P < 0.0001$). ROC analysis showed that serum PGE2 (area under the ROC curve, 0.83) could predict infection in patients with ACLF with sensitivity of 78.4% and specificity of 81.5% using a threshold of 141 pg/mL.
Conclusions: Serum PGE2 is associated with the susceptibility to secondary infections for patients with ACLF. Increased PGE2 serum levels may serve as a potential biomarker for developing infections in ACLF patients.

1. Introduction

Acute-on-chronic liver failure (ACLF) is a devastating syndrome characterized by acute deterioration of liver function and subsequent effects on multiple other organs [1–3]. Unfortunately, there is still no uniform accepted definition for the diagnosis of ACLF. Quite different from the diagnostic criteria for ACLF in western countries, the diagnostic criteria from the Asia-Pacific Association for the Study of the Liver (APASL) simply indicate that ACLF is diagnosed in the presence of an acute deterioration of a chronic liver disease [2]. In the diagnostic criteria of the APASL, total bilirubin (T-Bil) elevation and decreased prothrombin activity are essential for diagnosis. Chronic liver diseases that prelude ACLF also vary according to geographic region. In the Asia and Pacific region, chronic hepatitis B virus (HBV) infection represents 70% of the cases of ACLF [2,4].

Patients with ACLF are prone to develop infectious complications caused by bacteria and/or fungus, with a high risk of leading to the development of organ failure, prolonged hospital stay and increased mortality [5,6]. Therefore, early prediction of the infection risk and implementation of the appropriate immunomodulatory therapy to prevent infections could lead to better prognosis. As for the causes of high susceptibility to infections in patients with ACLF, an impaired immune response to microbial challenge termed as immune paralysis is thought to be crucial [5,7,8]. Furthermore, the decreased phagocytosis capacity and bacterial killing of both macrophages and neutrophils is paramount in the development

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Peer review under responsibility of Hainan Medical University.
Foundation Project: This study was supported by the National S&T Major Project (2017ZX10203201-002-002).

of immune paralysis in patients with ACLF [9–11]. The failure of the immune system to eliminate microbes increases the risk of developing secondary infections, which is both common and fatal in patients with ACLF. It has been postulated that the early diagnosis and prevention of infections could lead to better management of ACLF patients [12].

Prostaglandin E2 (PGE2) is a lipid mediator derived from arachidonic acid and is generated through an enzymatic cascade controlled by cyclooxygenase enzymes at sites of inflammation and infection [13]. It is well known that PGE2 has a variety of immunosuppressive functions including inhibition of macrophages phagocytosis and killing activity, neutrophils chemotaxis, production of proinflammatory mediators in leukocytes, and oxygen radical generation, thus resulting in the down regulation of immune functions and impairing host defense against microorganisms [14–18]. Recently, it was found that PGE2 plays a key role in the development of immune paralysis by suppressing proinflammatory cytokine secretion and bacterial killing of macrophages in patients with end-stage liver disease and acutely decompensated cirrhosis [19]. In view of the broad role of immunosuppressive functions mediated by PGE2, we hypothesized that PGE2 could also be a critical factor in the development of immune paralysis and infection in ACLF. Therefore, the present study was carried out to determine the serum levels of PGE2 in patients with ACLF and address its value as a potential predictive biomarker for developing infections.

2. Material and methods

2.1. Patients

From April 2014 to April 2015, ninety-one patients with HBV-ACLF but without infection were enrolled into this prospective study that was carried out at our Hospital. Twenty patients with stable chronic hepatitis B (CHB) were enrolled from the outpatient department and twenty healthy control subjects without any disease were enrolled from hospital staff. Patients were excluded if they showed any sign of infection within 72 h after admission.

This study was approved by the ethics committee of our Hospital. Each patient provided a written informed consent.

2.2. Diagnoses and data collection

The diagnosis of HBV-ACLF was made according to the APASL and Chinese Medical Association (CMA) guidelines. The CMA guidelines use higher cutoff levels of serum T-Bil (10 mg/dL) [20] than the APASL guidelines do (5 mg/dL) [5]. Patients with CHB were diagnosed based on the AASLD criteria [21]. Infection was diagnosed based on clinical symptoms (*e.g.*, fever, cough, expectoration, abdominal pain, and frequent, urgent, or painful urination), laboratory tests (*e.g.*, blood, sputum, ascites, and urine), and imaging findings (*e.g.*, chest X-ray and CT). The diagnosis of pneumonia was made by clinical signs and symptoms (*e.g.*, fever, emerging or worsening cough, purulent sputum, dyspnea, and tachycardia), radiographic findings (*e.g.*, the emergence of a new infiltrate or presence of a progressive infiltrate on chest radiography), and microbial data including GM test. The diagnosis of spontaneous bacterial peritonitis (SBP) was made in the presence of an ascitic fluid polymorphonuclear leukocytes count $\geq$250 cells/mm^3 with or without a positive culture of ascitic fluid [22]. Urinary tract infection (UTI) was diagnosed when a positive culture of urine ($\geq 10^5$ colonies/mL) was obtained with a urine polymorphonuclear leukocytes count >10 cells/mm^3 and associated clinical symptoms [23].

2.3. Follow-up

The follow-up lasted until the diagnosis of infection or 6 months, whichever occurred first.

2.4. PGE2 levels by ELISA

Blood (10 mL) was obtained from all included patients from an antecubital vein in a clotting tube. Blood samples were centrifuged at 3 000 rpm for 15 min at 4 °C to obtain serum. Samples were stored at −80 °C. Serum levels of PGE2 were quantified using a commercial ELISA kit (Invitrogen Inc., Carlsbad, CA, USA), according to the manufacturer's instructions. Intra-assay coefficient of variation (CV) was 3.5%–9.9% and inter-assay CV was 9.3%–15.9%.

2.5. Clinical data collection

Relevant clinical data were recorded when participants were enrolled. The basal clinical data and biochemical parameters were evaluated to assess the severity of liver disease. HBV DNA load was measured using a real-time polymerase chain reaction (PCR) system (Roche Diagnostics, Basel, Switzerland). Total white blood cell (WBC) count in peripheral blood and serum liver function, such as alanine transaminase (ALT), aspartate transaminase (AST), T-Bil, albumin (ALB), and PT were performed using standard clinical methods available at our hospital.

2.6. Statistical analysis

Data were presented as mean ± standard deviations (SDs) unless otherwise stated. One way ANOVA with post hoc test was used for analysis among multiple groups. The independent-samples t-test was used to compare the infection and no infection groups among patients with ACLF. Receiver operating characteristic (ROC) curves were used to obtain area under the curve (AUC) values and to determine optimal cut-off values to predict infection. SPSS 20.0 (IBM, Armonk, NY, USA) and GraphPad Prism 6.01 (GraphPad software Inc., San Diego, CA, USA) were used for analysis. Two-sided P-value <0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics

A total of 91 HBV-ACLF patients fulfilled the criteria for the study protocol during the study period. None of these patients had an infection when blood samples were collected for PGE2 analysis. Two groups of participants were enrolled for comparison: the first control group comprised 20 patients with stable CHB, while the second control group included 20 healthy controls. No significant difference was found among the ACLF, CHB, and control groups in terms of gender (P = 0.378) and age

(P = 0.072). The baseline characteristics of patients are presented in Table 1. ALT (P < 0.01), AST (P < 0.001), and TBil (P < 0.000 1) levels increased with worsening liver status, while ALB levels (P < 0.000 1) and PT (P < 0.000 1) decreased.

3.2. Comparison of serum PGE2 levels

Serum level of PGE2 was markedly higher in the ACLF group [(133.70 ± 7.71) pg/mL] compared with the CHB group [(26.15 ± 2.54) pg/mL] and the control group [(24.24 ± 2.30) pg/mL] (P < 0.000 1). There was no significant difference between patients with CHB and HCs (P = 0.58) (Table 1).

3.3. Association between PGE2 and risk of infection in the ACLF group

Among the 91 patients with HBV-ACLF, thirty-seven (40.7%) patients eventually developed secondary infections during follow-up, from which 33 were males and 4 were females. The mean age was (46.3 ± 9.6) years. Fifty-four (59.3%) patients did not develop secondary infections, from which 46 were males and 8 were females. The mean age was (43.6 ± 8.8) years. There were no significant differences regarding gender or age between these two groups (P > 0.05). WBC counts, HBV DNA, T-Bil, ALT, AST, ALB, and PT also showed no significant differences (P > 0.05) (Table 2).

Pulmonary infection was the most common infection, with 23 patients having significant pneumonia. Other types of infection were SBP (n = 12) and UTI (n = 2). The following pathogens were identified: *Aspergillus* (n = 11), *Escherichia coli* (n = 9), *Candida albicans* (n = 5), *Klebsiella pneumonia* (n = 4), *Enterococcus* (n = 3), *Staphylococcus* (n = 2), *Streptococcus* (n = 2), and one of unknown origin.

PGE2 levels were significantly elevated in patients with ACLF who developed an infection [(184.10 ± 11.34) pg/mL] compared with patients who showed no infectious events [(99.20 ± 7.39) pg/mL] (P < 0.000 1) (Table 2).

3.4. ROC analysis

ROC curve analyses confirmed that PGE2 was a significant predictor of secondary infections during admission for patients with ACLF with an AUC of 0.83 (95%*CI*: 0.75–0.92; P < 0.000 1), achieving a sensitivity of 78.4% and a specificity of 81.5% using a threshold of 141 pg/mL. The positive and negative predictive value was 0.787 and 0.871 respectively.

Table 2
Clinical features of HBV-ACLF patients who developed or not an infection.

Variables	No infection (n = 54)	Infection (n = 37)	P
Sex (M/F)	46/8	33/4	0.584 2
Age (years)	43.62 ± 8.83	46.25 ± 9.61	0.181 6
White blood cell (109/L)	5.53 ± 2.72	5.18 ± 2.58	0.655 5
HBV DNA (Log10)	5.67 ± 1.65	6.24 ± 1.88	0.129 8
ALT (U/L)	402.63 ± 589.74	436.09 ± 606.51	0.793 3
AST (U/L)	465.44 ± 636.48	510.73 ± 619.62	0.684 9
Total bilirubin (μmol/L)	395.24 ± 183.49	371.10 ± 131.66	0.493 5
Albumin (g/L)	29.25 ± 4.03	30.95 ± 4.28	0.679 8
Prothrombin activity (%)	30.51 ± 8.43	28.81 ± 9.69	0.376 4
PGE2 (pg/ml)	99.20 ± 7.39	184.10 ± 11.34	<0.000 1

Categorical variables are expressed as number and normal continuous variables as mean ± SD.

4. Discussion

Secondary infection is a common and severe complication in patients with ACLF. PGE2 could play a role in this high susceptibility of infections. Therefore, this study aimed to evaluate the serum PGE2 level in ACLF and to determine its predicative value for infection. Results showed that serum PGE2 was associated with the susceptibility to secondary infections for patients with ACLF. Increased PGE2 serum levels may serve as a potential biomarker for developing infections in ACLF patients.

In the present study, serum PGE2 levels were significantly higher in patients with ACLF compared with CHB as well as healthy controls. Similar results were observed by O'Brien *et al.* [19] and by Sarin *et al.* [24].

Most importantly, we identified for the first time that PGE2 was significantly higher in patients with ACLF who eventually developed secondary infections than those patients who did not, suggesting that serum PGE2 levels were significantly associated with the development of immune paralysis and secondary infections of ACLF. O'Brien *et al.* [19] also observed that treating patients with end-stage cirrhosis using albumin infusions could lower the PGE2 levels, attenuating the immune suppression, and reducing the risk of infections, supporting the present study. In a similar way, Brogliato *et al.* [25] used ketoprofen to treat patients with sepsis, inhibiting PGE2 production and alleviating the immunosuppression associated with sepsis.

Table 1
Baseline characteristics of participants.

Variables	Controls (n = 20)	CHB (n = 20)	ACLF (n = 91)	P
Gender (M/F)	15/5	16/4	79/12	0.378 0
Age (years)	39.50 ± 8.41	43.80 ± 9.24	44.7 ± 9.19	0.072 0
WBC	5.60 ± 1.82	5.80 ± 1.94	5.4 ± 2.65	0.881 5
HBV DNA (Log10)	–	6.21 ± 1.64	5.90 ± 1.76	0.472 1
ALT (U/L)	19.20 ± 8.46	258.27 ± 284.16	416.23 ± 593.49	<0.01
AST (U/L)	20.30 ± 10.61	137.62 ± 185.77	478.51 ± 626.78	<0.001
T-Bil (μmol/L)	18.47 ± 9.27	26.34 ± 29.82	385.42 ± 164.02	<0.000 1
ALB (g/L)	48.07 ± 3.41	46.43 ± 2.94	29.94 ± 4.19	<0.000 1
PTA (%)	–	97.72 ± 16.51	29.81 ± 8.95	<0.000 1
PGE2 (pg/ml)	24.24 ± 2.30	26.15 ± 2.54	133.70 ± 7.71	<0.000 1

Categorical variables are expressed as numbers and normal continuous variables as mean ± SD.

The ROC curve analysis showed the potential of PGE2 to predict secondary infections for patients with HBV-ACLF. Using a cut-off point of 141 pg/mL, PGE2 showed sensitivity of 78.4% and specificity of 81.5%. This successfully identified 29 out of 37 infection group patients and 44 out of 54 none-infection group patients after being followed up for 6 months. These results suggest that PGE2 could be used as a predicative marker for secondary infection of ACLF. However, since this is the first study reporting the role of PGE2 in predicting secondary infections in patients with ACLF, the best threshold for PGE2 and its role in predicting secondary infections in ACLF patients remain undetermined because of the limited number of patients. Therefore, before PGE2 can be routinely used for the management of ACLF, further studies are required using a larger subset of ACLF patients to evaluate the reliability.

Treatments aimed at inhibiting the immunosuppressive effect of PGE2 may prevent incidence of secondary infections and death from it for ACLF patients. It was demonstrated that the elevated level of PGE2 was associated with reduced serum albumin concentration in patients with acutely decompensated cirrhosis [19]. However, there was no significant difference in albumin levels between patients who developed infection and patients who did not go on to develop infection in the present study. These results suggest that the susceptibility to infections could be independent from liver function. Nevertheless, the present study was not designed to determine the exact mechanisms that could be responsible for these observations.

We assume that another possible cause for the elevation of PGE2 could be led by posttranscriptional changes of serum albumin (*e.g.*, dimers, oxidized isoform and N-terminal truncated isoform) [26,27]. Nevertheless, albumin levels and PGE2 levels are strongly associated [19,26,27]. This could be due to the impaired function of the liver in producing albumin and to the small sample size. Further studies of the association between the posttranscriptional changes of albumin and immune paralysis in patients with ACLF are required to evaluate the possibility using human albumin solution infusions to reduce circulating PGE2 level and attenuate immune suppression.

The present study is not without limitations. The sample size was small and from a single center. The follow-up was short (only 6 months). Finally, the study was not designed to determine the mechanisms leading to increased infection susceptibility. Additional studies are necessary to address these issues.

In conclusion, serum PGE2 was associated with the susceptibility to secondary infections for patients with ACLF. Increased PGE2 serum levels may serve as a potential biomarker for developing infections in ACLF patients.

Conflict of interest statement

We declare that we have no conflict of interest.

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