รายงานการวิจัย

เรื่อง

บทบาทของตัวบ่งชี้ทางระบบภูมิคุ้มกันที่เกี่ยวข้องกับการรักษาด้วยยาต้านไวรัสและการเกิดมะเร็งตับในผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบบีแบบเรื้อรัง

Role of immunological markers associated with treatment response and hepatocellular carcinoma development in patients with chronic hepatitis B virus

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บทคัดย่อ

B-cell activating factor (BAFF) เป็น cytokine ที่สำคัญในการกระตุ้นเซลล์เม็ดเลือดขาวชนิด B cell ที่เกี่ยวข้องกับการเกิดไวรัสตับอักเสบบี แต่อย่างไรก็ตามบทบาทของ BAFF ในผู้ป่วยมะเร็งตับที่เกิดจากการติดเชื้อไวรัสตับอักเสบบียังไม่ทราบแน่ชัด การศึกษานี้จึงมีวัตถุประสงค์ในการตรวจวัดระดับ BAFF ในพลาสมาและหาความสัมพันธ์กับความหลากหลายทางพันธุกรรมบนยีน BAFF rs9514828 และ rs12583006 และการทำนายและพยากรณ์ความรุนแรงของโรค โดยตรวจวัดระดับของ BAFF ในพลาสมาของผู้ที่มีสุขภาพดีกลุ่มควบคุม 100 คน กลุ่มผู้ป่วยที่มีการติดเชื้อไวรัสตับอักเสบบี 290 คน และกลุ่มผู้ป่วยมะเร็งตับจากการติดเชื้อไวรัสตับอักเสบบี 200 คน ผลการศึกษาพบว่าระดับของ BAFF ในพลาสมาในกลุ่มผู้ป่วยมะเร็งตับสูงกว่ากลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบบี และกลุ่มควบคุม ($P<0.001$). ระดับของ BAFF ในพลาสมายังมีความสัมพันธ์กับระดับ alpha-fetoprotein (AFP), ระยะของโรค (Child-Pugh classification), ขนาดของก้อนมะเร็ง (tumor size) และระยะของโรค (BCLC stage). เมื่อวิเคราะห์ปัจจัยเสี่ยงทั้งหมดด้วย Multivariate analyses พบว่าระดับของ BAFF (≥1,100 pg/ml) สามารถใช้ทำนายระยะเวลาการอยู่รอดของผู้ป่วยมะเร็งตับได้ (OR=2.28, 95%CI: 1.07–4.87; $P=0.034$). นอกจากนี้ยังพบว่าความหลากหลายทางพันธุกรรมบนยีน BAFF ตำแหน่ง rs9514828 พบความถี่ของจีโนไทป์ CT+TT ในกลุ่มผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบบีสูงกว่ากลุ่มควบคุม (58.0% vs. 46.0%, $P=0.029$). ดังนั้นผลการศึกษานี้จึงสรุปว่า ระดับ BAFF ในพลาสมาสูงมีความสัมพันธ์กับความรุนแรงและระยะเวลาการอยู่รอดของผู้ป่วยมะเร็งตับ เพราะฉะนั้นการทำงานของ B-cell อาจจะมีบทบาทสำคัญในการกระตุ้นการดำเนินโรคและพัฒนาเป็นมะเร็งตับ

Abstract

Abstract B-cell activating factor (BAFF), an important cytokine for B lymphocyte activation, has been shown to be increased in chronic hepatitis B virus (HBV) infection. This study aimed at evaluating clinical correlation and prognostic role of plasma BAFF and related polymorphisms in patients with hepatocellular carcinoma (HCC). Plasma BAFF levels were measured in samples of 100 healthy controls and 490 patients with chronic HBV infection (200 with HCC and 290 without HCC). The rs9514828 and rs12583006 polymorphisms were determined by allelic discrimination. The HCC group had significantly higher BAFF levels compared with the non HCC group and healthy controls ($P<0.001$) In HCC, elevated BAFF levels positively correlated with alpha-fetoprotein levels, Child-Pugh classification, tumor size and BCLC stage. Multivariate analyses showed that elevated BAFF (≥1,100 pg/ml) was an independent prognostic factor of overall survival in patients with HCC (OR=2.28, 95%CI: 1.07–4.87; $P=0.034$). Regarding BAFF polymorphisms, the frequency of rs9514828 CT+TT genotypes was higher distributed in patients with chronic HBV infection compared with healthy controls (58.0% vs. 46.0%, $P=0.029$). In summary, elevated BAFF levels at presentation correlated with disease severity and overall survival in patients with HCC, suggesting that B-cell immunity may play an essential role in promoting tumor development and progression.

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คำอธิบายสัญลักษณ์และคำย่อที่ใช้ในการวิจัย (List of Abbreviations)

BAFF, B cell-activating factor; HCC, hepatocellular carcinoma;

HBV, hepatitis B virus;

TNFSF13B, tumor necrosis factor superfamily;

AST, aspartate aminotransferase;

ALT, alanine aminotransferase;

AFP, alpha fetoprotein;

TB, total bilirubin;

SNPs, single nucleotide polymorphisms

ที่มาและความสำคัญ (Introduction)

การติดเชื้อไวรัสตับอักเสบบีเป็นปัญหาที่สำคัญทางสาธารณสุข เนื่องจากไวรัสตับอักเสบบีเป็นปัจจัยสำคัญของการเกิดตับอักเสบ การเกิดพังผืดในตับ ภาวะตับแข็ง และมะเร็งตับ [1] งานศึกษาวิจัยก่อนหน้านี้มีรายงานว่าพยาธิสภาพของการเกิดไวรัสตับอักเสบบีมีความสัมพันธ์กับภูมิคุ้มกันภายในร่างกาย [2] เมื่อเกิดภาวะติดเชื้อไวรัสแบบเฉียบพลัน ภูมิคุ้มกันของร่างกายโดยเฉพาะ B และ T-cell จะถูกกระตุ้นเพื่อต่อสู้กับไวรัส ในทางกลับกันหากเมื่อร่างกายติดเชื้อไวรัสตับอักเสบบีแบบเรื้อรัง ภูมิคุ้มกันของร่างกายมักจะทำงานผิดปกติไป [2] ในปัจจุบันพบว่า T-cell มีความสำคัญในการเกิดการทำลายของเซลล์ตับและการดำเนินโรค [3] อย่างไรก็ตามการศึกษาการทำงานของ ทั้ง T-cell และ B-cell ในผู้ป่วยติดเชื้อไวรัสตับอักเสบบีแบบเรื้อรังยังคงมีการศึกษาไม่มาก

B cells มีหน้าที่สำคัญในการผลิตภูมิคุ้มกันหรือแอนติบอดี้ ควบคุมการหลั่งสารอักเสบ และกระตุ้นการทำงานของ T-cell [4,5] ซึ่งโดยทั่วไปการทำงานของเม็ดเลือดขาว (B lymphocytes) จำเป็นต้องอาศัย B cell-activating factor (BAFF) ซึ่งเป็นโปรตีนที่อยู่ในกลุ่มของ tumor necrosis factor superfamily (TNFSF13B) และ IFN stimulated gene และสามารถพบได้จากการหลั่งของเม็ดเลือดขาวชนิด neutrophils, และ monocytes เซลล์แมคโคฟาจ (macrophages) dendritic cells และ T cells ที่ถูกกระตุ้น [6] การศึกษาก่อนหน้านี้พบว่าระดับของ BAFF ที่สูงขึ้นมีความสัมพันธ์กับการดำเนินโรคที่แย่ลง [7-9] นอกจากนี้ยังพบว่ามีความสัมพันธ์กับการเกิดมะเร็งบางชนิด [10] นอกจากนี้ยังพบสูงในคนไข้ไวรัสตับอักเสบซีแบบเรื้อรังโดยเฉพาะในกลุ่มที่มี cryoglobulinemia อีกด้วย [11,12] และในผู้ป่วยไวรัสตับอักเสบบีซึ่งมีความเสี่ยงต่อการเกิดมะเร็งตับอีกด้วย [13] ข้อมูลนี้จึงอาจสรุปได้ว่าระดับของ BAFF อาจส่งผลให้เกิดการดำเนินโรคตับ และพัฒนาไปเป็นมะเร็งตับในผู้ป่วยไวรัสตับอักเสบบีแบบเรื้อรังได้ในอนาคต อย่างไรก็ตามความสัมพันธ์ของ BAFF กับผลทางคลินิกและการพัฒนาเป็นมะเร็งตับยังไม่มีการศึกษาได้มากเท่าที่ควร

ความหลากหลายทางพันธุกรรมหรือ single nucleotide polymorphisms (SNPs) บนยีน *BAFF* ประกอบด้วยตำแหน่ง rs9514828 และ rs12583006 ซึ่งมีความสัมพันธ์กับระดับของ BAFF และโรคเกี่ยวกับระบบภูมิคุ้มกันบกพร่องและโรคเลือด [14-16] แต่ยังพบการศึกษาน้อยในผู้ป่วยไวรัสตับอักเสบบี [17] และยังไม่ทราบว่า SNPs ตำแหน่งดังกล่าวมีความสัมพันธ์กับความรุนแรงและการเกิดมะเร็งตับหรือไม่ เพราะฉะนั้นการศึกษานี้จึงศึกษาหาความสัมพันธ์ของระดับของ BAFF ในพลาสมากับ SNPs และผลทางคลินิกในผู้ป่วยมะเร็งตับที่ได้รับการติดเชื้อจากไวรัสตับอักเสบบี

วิธีดำเนินการวิจัย (Materials & Method)

1.การเก็บตัวอย่างที่ใช้ในงานวิจัย

เก็บตัวอย่างเลือดเพื่อใช้ในการตรวจวิเคราะห์ระดับของ BAFF และ ความหลากหลายทางพันธุกรรมของยีน BAFF จากผู้ป่วยโรคมะเร็งตับที่มีความสัมพันธ์กับการติดเชื้อไวรัสตับอักเสบบีของโรงพยาบาลจุฬาลงกรณ์ซึ่งยืนยันผลการติดเชื้อไวรัสตับอักเสบบีจากผลการตรวจค่า HBsAg ในเลือดและยืนยันผลของมะเร็งจากการอ่านผลพยาธิวิทยาของชิ้นเนื้อตับ

โดยแบ่งเป็นผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบบีแบบเรื้อรังที่พบและไม่พบมะเร็งตับและคัดผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซี ผู้ป่วยติดเชื้อ HIV และผู้ป่วยที่เป็นมะเร็งชนิดอื่นออก

2.การตรวจทางห้องปฏิบัติการ

การตรวจเชิงคุณภาพของ HBsAg, HBeAg จากซีรั่ม ด้วย enzyme-linked immunosorbent assays ระดับ HBV DNA ด้วย Abbott Real Time HBV assay และตรวจระดับ BAFF จากพลาสมาโดยใช้เทคนิค ELISA

3.การศึกษาจีโนไทป์ของ BAFF

สกัดดีเอ็นเอจาก peripheral blood mononuclear cells (PBMC) 100 ไมโครลิตร ด้วยวิธี phenol-chloroform ตรวจเชิงคุณภาพของดีเอ็นเอด้วยสเปกโตรโฟโตมิเตอร์ (NanoDrop 2000c, Thermo Scientific) และตรวจจีโนไทป์ rs9514828 ด้วยวิธีการ PCR โดยใช้ PCR master mix (Thermo scientific)

ไพรเมอร์ : 5’-GGCACAGTCAACATGGGAGT-3’(forward)

5’-GCTAAGTGTTTTAGCATTGAATTG-3’ (reverse)

จากการศึกษา [15] จะใช้สภาวะที่เหมาะสมดังนี้

initial denaturation at 95 องศาเซลเซียส เป็นเวลา 3 นาที

95 องศาเซล เซียส เป็นเวลา 30 วินาที
58 องศาเซลเซียส เป็นเวลา 30 วินาที } 40 รอบ
72 องศาเซลเซียส เป็นเวลา 1 นาที

final extension 72 องศาเซลเซียส เป็นเวลา 7 นาที

ตัดย่อยผลิตภัณฑ์ที่ได้ด้วยเอนไซม์ BSrBI และติดตามผลด้วย 2 เปอร์เซ็นอะกาโรสเจลอิเล็กโทรโฟลิซิส

ส่วน BAFF จีโนไทป์ rs12583006 จะใช้ TaqMan genotyping assay (C_11705495_10, Applied Biosystem) โดยใช้ TaqMan genotyping master mix (Applied Biosystems) 20X primers และprobes mixture (TaqMan SNP Genotyping Assay, Applied Biosystems) ด้วย ABI 7500 Real Time PCR System (Applied Biosystems)

จะใช้สภาวะที่เหมาะสมดังนี้

initial denaturation at 95 องศาเซลเซียส เป็นเวลา 10 นาที

denaturation 95 องศาเซลเซียส เป็นเวลา 15 วินาที
annealing 60 องศาเซลเซียส เป็นเวลา 1 นาที
extension 60 องศาเซลเซียส เป็นเวลา 1 นาที
} 40 รอบ

4.การวิเคราะห์ข้อมูลและสถิติที่ใช้วิเคราะห์

ใช้โปรแกรม SPSS version 22.0 ในการวิเคราะห์ผลการวิจัยโดยนำเสนอข้อมูลในรูปค่าเฉลี่ยและส่วนเบี่ยงเบนมาตรฐาน เปรียบเทียบความสัมพันธ์ระหว่างกลุ่มของตัวแปรด้วย chi-square หรือ Student’s t-test ค่า $p < 0.05$ จะถูกพิจารณาว่ามีความแตกต่างอยางมีนัยสำคัญทางสถิติ

อภิปรายผล (Discussion)

มะเร็งตับเป็นมะเร็งที่มีความชุกและพบมากในเอเชียตะวันออกเฉียงใต้ [1] โดยทั่วไปการทำนายโรคมะเร็งตับนั้นทำได้ยาก เนื่องจากคนไข้มักมาพบแพทย์ในระยะท้ายแล้วเนื่องจากมะเร็งตับที่เกิดจากการติดเชื้อไวรัสตับอักเสบบี มีปัจจัยหลายอย่างโดยเฉพาะอย่างยิ่งภูมิคุ้มกันอ่อนแอส่งผลให้ตับถูกทำลาย [2] รายงานการศึกษาก่อนหน้านี้พบว่าระบบภูมิคุ้มกันที่มีความจำเพาะเจาะจง (specific immune system) ต่อชนิดของเชื้อโรค หรือ Adaptive immune โดยเฉพาะอย่างยิ่ง T-cells มีบทบาทสำคัญต่อการอักเสบของตับ รวมไปถึงการเกิดมะเร็งตับ [22] ในทางกลับกันข้อมูลการศึกษาการทำงานของ B-cell ในการทำนายการดำเนินโรคมะเร็งตับยังมีการศึกษาไม่เพียงพอ

การศึกษานี้ให้ผลสอดคล้องกับงานวิจัยก่อนหน้านี้ โดยพบว่าระดับของ BAFF สูงขึ้นอย่างมีนัยสำคัญทางสถิติในผู้ป่วยมะเร็งตับเมื่อเปรียบเทียบกับผู้ที่มีสุขภาพดีและผู้ป่วยไวรัสตับอักเสบบีแบบเรื้อรัง [13] เมื่อพิจารณากลุ่มผู้ป่วยไวรัสตับอักเสบบี พบว่าระดับของ BAFF เพิ่มขึ้นในกลุ่มผู้ป่วย inactive carriers (IC), immune active (IA) และตับแข็งตามลำดับ ผลการศึกษาพบว่าระดับของ BAFF สัมพันธ์กับระดับของ ALT ที่เป็นตัวบ่งชี้การทำลายของเซลล์ตับ ผลการศึกษานี้สรุปได้ว่าระดับของ BAFF ในพลาสมาเพิ่มขึ้นเมื่อมีการอักเสบแบบเฉียบพลัน ซึ่งอาจเป็นไปได้ว่าเกิดจากการกระตุ้น type I interferons [4] โดยเฉพาะอย่างยิ่งในกลุ่มผู้ป่วย immune active โดยพบว่าระดับของ BAFF ในผู้ป่วยไวรัสตับอักเสบบีที่มี HBeAg-positive สูงกว่าผู้ป่วย HBeAg-negative ซึ่งสอดคล้องกับผลการทดลองในเซลล์เพาะเลี้ยงที่พบว่า HBeAg สามารถกระตุ้นการทำงานของ BAFF ผ่านทางการทำงานของ monocyte [22] นอกจากนี้ยังพบว่าระดับของ BAFF มีความสัมพันธ์กับการเกิดพังผืดตับ (FIB-4 index) และมีระดับของ BAFF ในกลุ่มผู้ป่วยที่มีภาวะตับแข็งสูงกว่าผู้ป่วยที่ไม่มีภาวะตับแข็ง ผลการศึกษานี้สนับสนุนการศึกษาก่อนหน้านี้ว่าระดับของ BAFF มีความสัมพันธ์กับการเกิดภาวะตับแข็งและพบมากขึ้นตามสาเหตุของการเกิดโรคตับ [21,23]

ผลการศึกษานี้พบว่าระดับของ BAFF ที่เพิ่มสูงขึ้นในพลาสมามีความสัมพันธ์กับขนาดของก้อนมะเร็งและความรุนแรงของโรคมะเร็งตับในผู้ป่วยมะเร็งตับที่เกิดจากการติดเชื้อไวรัสตับอักเสบบี นอกจากนี้ระดับของ BAFF ในพลาสมาสูงมักจะพบในผู้ป่วยมะเร็งตับที่มีก้อนขนาดใหญ่ และอยู่ในระยะรุนแรง นอกเหนือจากนี้การวิเคราะห์ปัจจัยหลายปัจจัยร่วมกัน (multivariate analysis) พบว่า BAFF สามารถใช้ทำนายอัตราการอยู่รอดชีวิตของผู้ป่วยมะเร็งตับได้ ผู้ป่วยที่มีระดับตั้งต้นของ BAFF มากกว่า 1,100 pg/ml มีความเสี่ยงต่อความรุนแรงของโรคมากกว่าผู้ป่วยที่มีระดับ BAFF ต่ำ ข้อมูลนี้ให้ผลยืนยันว่าอาจใช้ระดับของ BAFF ในการทำนายความรุนแรงของโรคมะเร็งตับได้ และอาจใช้เป็นตัวบ่งชี้ทางชีวภาพที่ใช้ติดตามการเจริญของก้อนมะเร็งและใช้ทำนายความรุนแรงของโรคในผู้ป่วยมะเร็งตับที่เกิดจากการติดเชื้อไวรัสตับอักเสบบี

เช่นเดียวกับกับการศึกษานี้ที่พบว่าระดับของ BAFF มีความสัมพันธ์กับก้อนมะเร็งและความรุนแรงของโรคในผู้ป่วย hematological และ non-hematological malignancies [9, 24-27] นอกจากนี้ระดับของ BAFF ยังมีความสัมพันธ์กับโรค multiple myeloma [26] และยังพบสูงในผู้ป่วยมะเร็งตับอ่อนโดยเฉพาะในผู้ป่วยที่มีการแพร่กระจายของเซลล์มะเร็งแล้ว [26] ผลการศึกษานี้พบว่าบทบาทของ BAFF มีความเกี่ยวข้องกับความรุนแรงของโรคมะเร็งตับและมะเร็งหลายชนิด

5

BAFF เป็นปัจจัยสำคัญของการเจริญของ B cells ซึ่งอาจจะมีผลกับการดำเนินโรคและการพัฒนาเป็นมะเร็งตับในผู้ป่วยโรคไวรัสตับอักเสบบี ซึ่งผลการศึกษาก่อนหน้านี้พบว่าการทดลองในหนูที่ไม่มี B cell ถูกกระตุ้นให้มีพังผืดในตับได้จากการกระตุ้น CCl_4 ซึ่งเป็น profibrogenic ที่กระตุ้นการทำงานของ B cell [28] การศึกษาเร็วๆนี้ พบว่า B cells ภายในตับส่งผลให้เกิดพังผืดได้ โดยการกระตุ้น hepatic stellate cell และการสร้างสารอักเสบ (inflammatory cytokines) [29] นอกจากนี้ยังมีบทบาทสำคัญในการมีคุณสมบัติกระตุ้นให้เกิดมะเร็งตับ [30] ในหนูทดลองที่ไม่มี B cells แต่มี T cells พบว่าสามารถป้องกันการพัฒนาเป็นมะเร็งตับได้ นอกจากนี้ผลการศึกษาในผู้ป่วยมะเร็งตับพบว่า B cells ที่เพิ่มขึ้นมีความสัมพันธ์กับการเจริญของก้อนมะเร็ง และผู้ป่วยมีระยะเวลาอยู่รอดลดลง [30] เช่นเดียวกับ B cells ที่เพิ่มขึ้นใน PBMCs ในผู้ป่วยมะเร็งตับระยะรุนแรงมากกว่าผู้ป่วยมะเร็งตับระยะเริ่มต้น [25] เพราะฉะนั้นนอกจาก B cells จะควบคุมการเกิดพังผืดตับยังมีความสัมพันธ์กับการพัฒนาไปเป็นมะเร็งตับอีกด้วย

ความหลากหลายทางพันธุกรรมบนยีน BAFF อาจส่งผลต่อการแสดงออกของ BAFF และมีความสัมพันธ์กับพยาธิสภาพของโรคภูมิคุ้มกัน โรคมะเร็งเลือด หรือโรคติดเชื้อเรื้อรัง [14-16] การศึกษาก่อนหน้านี้พบว่า T allele ของ SNPs ตำแหน่ง rs9514828 พบมาในผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบซีร่วมกับ cryoglobulinemia (MC) และมีความสัมพันธ์กับระดับของ BAFF ที่สูงขึ้นเมื่อเปรียบเทียบกับผู้ป่วยไวรัสตับอักเสบซีที่ไม่มี MC [31, 32] ในการศึกษานี้พบว่าความถี่ของ CT+TT genotype บน SNPs ตำแหน่ง rs9514828 พบมากในกลุ่มผู้ป่วยไวรัสตับอักเสบบีและผู้ที่เป็นมะเร็งตับร่วมด้วยสูงกว่ากลุ่มควบคุมผู้ที่มีสุขภาพดี อย่างไรก็ตามไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ยังไม่พบความสัมพันธ์กับระดับของ BAFF และผลทางคลินิกอื่นๆ ผลการศึกษานี้อาจสรุปได้ว่า CT+TT genotype ที่พบอาจมีความสัมพันธ์กับการติดเชื้อไวรัสตับอักเสบบีแต่ไม่มีความสัมพันธ์กับการพัฒนาเป็นมะเร็งตับในประชากรชาวไทย ซึ่งสอดคล้องกับการศึกษาในคนจีนชาวฮั่น [17] ส่วน SNPs ตำแหน่ง rs12583006 ผลการศึกษานี้พบว่าความหลากหลายทางพันธุกรรมนี้ไม่มีความสัมพันธ์กับระดับของ BAFF ในพลาสมาเช่นกัน และที่สำคัญไม่มีความสัมพันธ์กับการติดเชื้อไวรัสตับอักเสบบีอีกด้วย

สรุปและเสนอแนะการวิจัยขั้นต่อไป

การศึกษานี้อาจมีข้อจำกัด เนื่องจากเป็นการศึกษาแบบย้อนหลังและมีขนาดของประชากรที่ศึกษาน้อย การศึกษาความหลากหลายทางพันธุกรรมเพียง 2 ตำแหน่งบนยีนนี้เป็นการศึกษาเฉพาะในประชากรไทยซึ่งไม่สามารถใช้กับประชากรเชื้อชาติอื่น การศึกษานี้จึงสรุปได้ว่า การศึกษานี้เป็นการศึกษาแรกที่ศึกษาความสัมพันธ์ของระดับของ BAFF ในพลาสมาของผู้ป่วยมะเร็งตับที่เกิดจากการติดเชื้อไวรัสตับอักเสบบี และสามารถประยุกต์ใช้ทางคลินิกได้ โดยพบว่าระดับของ BAFF ที่สูงขึ้นมีความสัมพันธ์กับขนาดของก้อนมะเร็งและความรุนแรงของโรค นอกจากนี้ยังอาจใช้ระดับของ BAFF เป็นตัวบ่งชี้ทางชีวภาพที่ใช้ในการวินิจฉัย และทำนายการอยู่รอดของผู้ป่วยมะเร็งตับได้ และอาจสรุปได้ว่าระบบภูมิคุ้มกันและการทำงานของ B cell มีความสัมพันธ์กับการดำเนินโรคและพัฒนาเป็นมะเร็งตับในผู้ป่วยที่ติดเชื้อไวรัสตับอักเสบบีแบบเรื้อรังได้ อย่างไรก็ตามคงต้องมีการศึกษาหรือพิสูจน์การทำงานของ B-cell ที่เกี่ยวข้องกับระบบภูมิคุ้มกันที่นำไปสู่การเกิดมะเร็งตับต่อไป

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ภาคผนวก (Appendix)

ตารางที่1 คุณสมบัติของอาสาสมัครที่เข้าร่วมการศึกษา

Baseline Characteristics	Healthy controls (n = 100)	Patients without HCC (n = 290)	Patients with HCC (n = 200)	*P*
Age (years)	49.3 ± 5.2	42.9 ± 11.8	58.1 ± 11.9	< 0.001*
Gender (Male)	65 (65.0)	174 (60.0)	168 (84.0)	< 0.001*
Aspartate aminotransferase (IU/L)		39.6 ± 35.9	95.6 ± 102.2	< 0.001*
Alanine aminotransferase (IU/L)		58.9 ± 70.3	59.5 ± 54.3	0.915
Serum albumin (g/dL)		4.4 ± 0.4	3.6 ± 0.6	< 0.001*
Total bilirubin (mg/dL)		0.7 ± 0.3	1.2 ± 0.7	< 0.001*
Platelet count (10^9/L)		228.6 ± 54.4	200.0 ± 126.9	0.003*
HBeAg positivity		95 (33.0)	58 (29.0)	0.468
Log10 HBV DNA (IU/mL)		4.8 ± 2.2	4.5 ± 1.5	0.199
Alpha fetoprotein (ng/mL)		5.3 ± 14.5	17203.5 ± 60745.5	0.007*
FIB-4 index		1.26 ± 0.83	4.87 ± 4.14	< 0.001*
Presence of cirrhosis		52 (17.9)	168 (84.0)	< 0.001*
BCLC stage (0-A/B/C-D)		-	61(30.5)/76(38.0)/3(31.5)	-

ตารางที่2 ความสัมพันธ์ระหว่างระดับ BAFF ในพลาสมากับคุณสมบัติของอาสาสมัคร

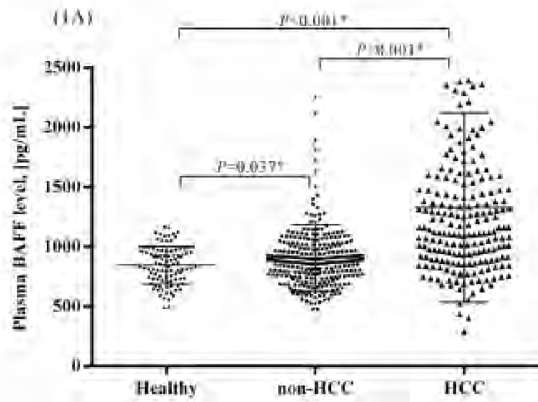
Variables	Low BAFF (< 1100 pg/ml) (n = 99)	High BAFF (≥ 1100 pg/ml) (n = 101)	*P*
Age (years)	58.2 ± 11.9	58.0 ± 11.9	0.900
Gender			0.177
Male (n = 168)	87 (87.9)	81 (80.2)	
Female (n = 32)	12 (12.1)	20 (19.8)	
Aspartate aminotransferase (IU/L)	72.4 ± 71.8	118.4 ± 121.1	0.001*
Alanine aminotransferase (IU/L)	59.3 ± 17.5	59.8 ± 51.2	0.954
Serum albumin (g/dL)	3.8 ± 0.6	3.4 ± 0.5	< 0.001*
Total bilirubin (mg/dL)	1.0 ± 0.6	1.3 ± 0.8	0.008*
Platelet count (10^9/L)	188.8 ± 121.7	210.9 ± 131.5	0.221
Log10 HBV DNA (IU/mL)	4.5 ± 1.5	4.4 ± 1.5	0.879
Alpha fetoprotein (ng/mL)	5210.3 ± 16801.2	28735.4 ± 8208.0	0.016*
FIB-4 index	4.26 ± 3.92	5.46 ± 4.28	0.069
Child-Puge class			
A (n = 158)	89 (87.3)	69 (70.4)	0.027*
B or C (n = 42)	13 (12.7)	29 (29.6)	
BCLC tumor stage			
0-A (n = 61)	40 (40.4)	21 (20.8)	< 0.001*
B (n = 76)	41 (41.4)	35 (34.7)	
C-D (n = 63)	18 (18.2)	45 (44.6)	

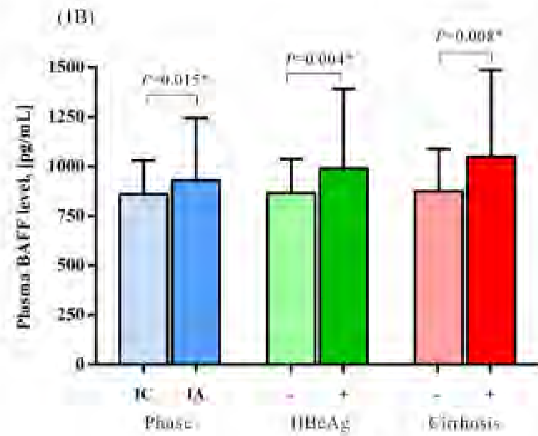
ตารางที่3 ความชุกของความหลากหลายทางพันธุกรรม

Polymorphisms	Healthy controls (n = 100)	Patients without HCC (n = 290)	Patients with HCC (n = 200)	Patients with and without HCC (n = 490)	HCC vs. Healthy controls		HCC vs. Non-HCC		Non-HCC and HCC vs. Healthy controls	
					OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs9514828										
Genotype frequency										
CC	54 (54.0)	116 (40.0)	90 (45.0)	206 (42.0)	1.10		1.00		1.00	
CT	36 (36.0)	142 (49.0)	90 (45.0)	232 (47.4)	1.50 (0.90-2.51)	0.121	0.82 (0.56-1.20)	0.299	1.69 (1.06-2.68)	0.026*
TT	10 (10.0)	32 (11.0)	20 (10.0)	52 (10.6)	1.20 (0.52-2.75)	0.667	0.81 (0.43-1.50)	0.496	1.36 (0.65-2.86)	0.412
CT + TT	46 (46.0)	174 (60.0)	110 (55.0)	284 (58.0)	1.43 (0.89-2.32)	0.142	0.81 (0.57-1.17)	0.271	1.62 (1.05-2.49)	0.029*
Allele frequency										
C	144 (72.0)	374 (64.5)	270 (67.5)	644 (65.7)	1.10		1.00		1.00	
T	56 (28.0)	206 (35.5)	130 (32.5)	336 (34.3)	1.24 (0.85-1.80)	0.261	0.87 (0.67-1.14)	0.328	1.34 (0.96-1.88)	0.086
rs12583006										
Genotype frequency										
AA	19 (19.0)	55 (19.0)	36 (18.0)	91 (18.6)	1.10		1.00		1.00	
AT	42 (42.0)	147 (50.7)	87 (43.5)	234 (47.8)	1.09 (0.56-2.13)	0.793	0.90 (0.55-1.49)	0.691	1.16 (0.64-2.11)	0.618
TT	39 (39.0)	88 (30.3)	77 (38.5)	165 (33.6)	1.04 (0.53-2.05)	0.905	1.34 (0.80-2.25)	0.274	0.88 (0.48-1.62)	0.688
AT + TT	81 (81.0)	235 (81.0)	164 (82.0)	399 (81.4)	1.07 (0.58-1.98)	0.833	1.07 (0.67-1.70)	0.787	1.03 (0.59-1.78)	0.920
Allele frequency										
A	80 (40.0)	257 (44.3)	159 (39.8)	416 (42.4)	1.10		1.00		1.00	
T	120 (60.0)	323 (55.7)	241 (60.2)	564 (57.6)	1.01 (0.71-1.43)	0.953	1.21 (0.93-1.56)	0.156	0.90 (0.66-1.23)	0.523

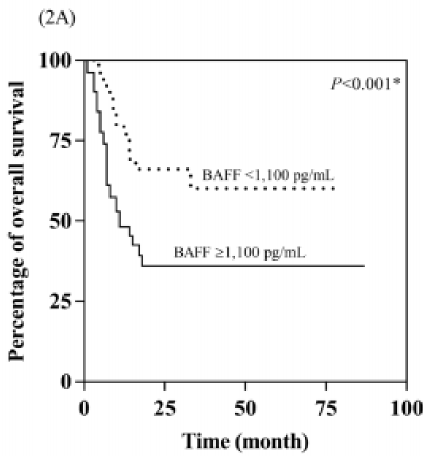
Factors	Category	Overall survival			
		Univariate analysis		Multivariate analysis	
		OR (95%CI)	*P*	OR (95%CI)	*P*
Age (years)	< 60 vs. ≥ 60	1.98 (1.14-3.45)	0.016*	0.69 (0.30-1.60)	0.387
Gender	Male vs. Female	1.35 (0.70-2.61)	0.374		
Aspartate aminotransferase (IU/L)	< 60 vs. ≥ 60	3.14 (1.76-5.63)	< 0.001*	0.92 (0.39-2.19)	0.853
Alanine aminotransferase (IU/L)	< 60 vs. ≥ 60	2.74 (1.62-4.65)	< 0.001*	1.34 (0.63-2.82)	0.445
Platelet count (10^9/L)	≥ 150 vs. < 150	2.94 (1.55-5.57)	0.001*	1.94 (0.75-5.02)	0.174
Log10 HBV DNA (IU/mL)	< 4.0 vs. ≥ 4.0	0.78 (0.39-1.56)	0.475		
Child-Pugh classification	A vs. B and C	1.42 (0.67-3.02)	0.361		
Alpha fetoprotein (ng/mL)	< 100 vs. ≥ 100	5.91 (2.99-11.68)	< 0.001*	3.64 (1.53-8.64)	0.003*
FIB-4 index	< 3.40 vs. ≥ 3.40	0.87 (0.52-1.51)	0.656		
Tumor size (cm.)	< 5.0 vs. ≥ 5.0	10.55 (4.69-23.75)	< 0.001*	2.10 (0.62-7.10)	0.231
BCLC stage	0, A vs. B, C, D	4.42 (2.91-6.70)	< 0.001*	3.00 (1.53-5.87)	0.001*
Plasma BAFF level (pg/ml)	< 1100 vs. ≥ 1100	3.10 (1.75-5.49)	< 0.001*	2.28 (1.07-4.87)	0.034*
rs9514828	CC vs. CT + TT	1.32 (0.52-3.32)	0.557		
rs12583006	AA vs. AT + TT	0.98 (0.57-1.67)	0.931		

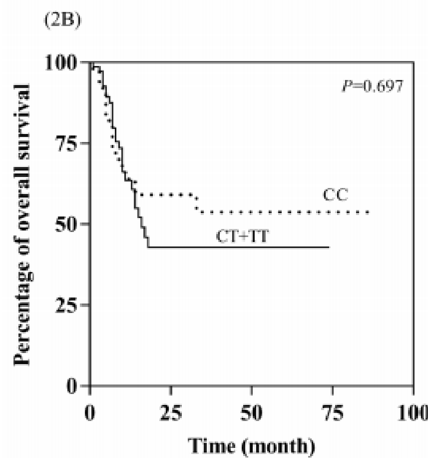
ตารางที่4 ปัจจัยที่มีความเกี่ยวข้องกับการรอดชีวิตของผู้ป่วยมะเร็งตับ

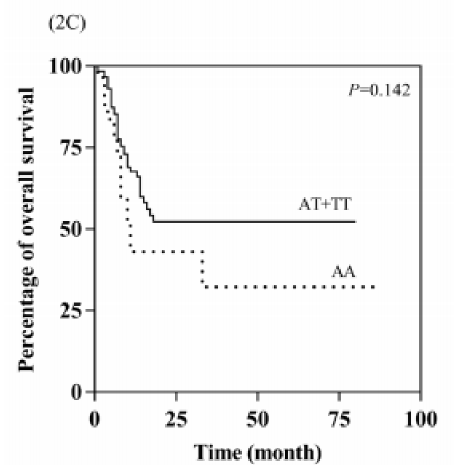




รูปภาพที่1 ระดับของ BAFF ในพลาสมา 1A) แต่ละกลุ่มของผู้ป่วยและอาสาสมัครสุขภาพดี 1B) กลุ่มย่อยของผู้ป่วยติดเชื้อไวรัสตับอักเสบบีที่ไม่เป็นมะเร็งตับ







รูปภาพที่2 อัตราการรอดชีวิตทั้งหมดของผู้ป่วยมะเร็งตับที่เกี่ยวข้องกับระดับของ BAFF และความหลากหลายทางพันธุกรรม A)ระดับของ BAFF ในพลาสมา B)จีโนไทป์ rs9514828 C)จีโนไทป์ rs12583006

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Pathogenesis and non-invasive diagnostic tests of hepatic fibrosis and cirrhosis

Pathogenesis, tumor markers and treatment of hepatocellular carcinoma

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International Publications

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3. The Hepato Biliary and Pancreatic Surgery Society of Thailand

Publications

1. Radiation enteritis and proctitis. Chula Surgical Proceeding 1992; 8 (5): 190-208.
2. Gastric leiomyosarcoma. Chula Surgical Proceedings 1993; 9(3): 125-136.
3. Malrotation. Chula Surgical Proceedings 1993; 9 (2): 42-45.
4. Salivary gland tumor. Chula Surgical Proceedings. 1993; 9 (6): 236-246.
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2000 : Organizing Committee of Asean Medical Student Association Meeting in Thailand.

2004 : Winning the Top-score Award of the Physics Examination, The Radiological Society of Thailand

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PROFESSIONAL TRAINING

2003-2004 : Internship as General Practitioner at Prince at Prince of Songkhla University Hospital, Songkhla, Thailand

2004-2007 : Resident in Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

2011-2012 : Fellowship of Interventional Oncology/Radiology, Dotter Institutes, Oregon Health and Sciences University Hospital, Oregon, USA

2007-present : Working as Instructor/Attending physician in Vascular and Interventional Radiology Unit, King Chulalongkorn Memorial Hospital, Bangkok, Thailand

CERTIFICATE&LICENSURES

2003 : Doctor of Medicine Diploma and certificate of Licensure, ChulalongkornUniversity and Thai Medical Council

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2012 : Certificate in Fellowship of Interventional Oncology/Radiology, Dotter Institutes, Oregon, USA

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MEMBERSHIP IN PROFESSIONAL ASSOCIATIONS

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PUBLICATIONS

1. Sriprapun M, Chuaypen N, Khlaiphuengsin A, **Pinjaroen N**, Payungporn S, Tangkijvanich P. Association of PINX1 but not TEP1 Polymorphisms with Progression to Hepatocellular Carcinoma in Thai Patients with Chronic Hepatitis B Virus Infection. Asian Pac J Cancer Prev. 2016;17(4):2019-25. PubMed PMID: 27221889.

2. Chanthra N, Payungporn S, Chuaypen N, Piratanantatavorn K, **Pinjaroen N**, Poovorawan Y, Tangkijvanich P. Single Nucleotide Polymorphisms in STAT3 and STAT4 and Risk of Hepatocellular Carcinoma in Thai Patients with Chronic Hepatitis B. Asian Pac J Cancer Prev. 2015;16(18):8405-10. PubMed PMID: 26745093.

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- Travel Award for Outstanding Young Investigator [Asian Pacific Digestive Week 2016 (APDW 2016) Kobe, Japan, 2016]
- Young Investigator Award [25th Asian Pacific Association for the Study of the Liver (APASL), Tokyo, Japan, 2016]
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International Publications:

1. **Chuaypen N**, Tuyapala N, Pinjaroen N, Payungporn S, Tangkijvanich P. Association of NTCP polymorphisms with clinical outcome of hepatitis B infection in Thai Individuals (under review).
2. **Chuaypen N**, Chittmittraprap S, Pinjaroen N, Sirichindakul B, Poovorawan Y, Tanaka Y, Tangkijvanich P. Serum WFA+-M2BP Level as a Diagnostic Marker of Hepatitis B Virus-related Hepatocellular Carcinoma. Hepatol Res 2018 May 6. doi: 10.1111/hepr.13187. [Epub ahead of print] (Q2, IF=3.415).
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5. Kiatbumrung R, **Chuaypen N**, Payungporn S, Avihingsanon A, Tangkijvanich P. The association of PNPLA3, COX-2 and DHCR7 polymorphisms with advanced liver fibrosis in patients with HCV mono-infection and HCV/HIV co-infection. Asian Pac J Cancer Prev 2018.
6. **Chuaypen N**, Payungporn S, Tangkijvanich P. Next generation sequencing identifies baseline HBV mutants associated with treatment response to pegylated interferon in patients with HBeAg-positive chronic hepatitis B (under review).
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9. Limothai U, **Chuaypen N**, Khlaiphuengsin A, Chittmittraprap S, Poovorawan Y, Tangkijvanich P. Association of vitamin-D-related genetic variations and treatment response to pegylated interferon in patients with chronic hepatitis B. Antivir Ther 2017; 22: 681-8. (Q2=2.146).

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14. Jinato T, **Chuaypen N**, Poomipak W, Praianantathavorn K, Makkoch J, Kiatbumrung R, et al. Analysis of hepatic microRNA alterations in response to hepatitis B virus infection and pegylated interferon alpha-2a treatment. Exp Biol Med (Maywood) 2016; 241: 1803-10. (Q1, IF=2.413).
15. Sriprapun M, **Chuaypen N**, Khlaiphuengsin A, Pinjaroen N, Payungporn S, Tangkijvanich P. Association of PINX1 but not TEP1 polymorphisms with progression to hepatocellular carcinoma in Thai patients with chronic hepatitis B virus Infection. Asian Pac J Cancer Prev 2016;17:2019-25.
16. Makkoch J, Praianantathavorn K, Sopipong W, **Chuaypen N**, Tangkijvanich P, Payungporn S. Genetic variations in XRCC4 (rs1805377) and ATF6 (rs2070150) are not associated with hepatocellular carcinoma in Thai patients with hepatitis B virus infection. Asian Pac J Cancer Prev 2016;17:591-5.
17. Chanthra N, Payungporn S, **Chuaypen N**, Pinjaroen N, Poovorawan Y, Tangkijvanich P. Association of single nucleotide polymorphism rs1053004 in signal transducer and activator of transcription 3 (STAT3) with susceptibility to hepatocellular carcinoma in Thai patients with chronic hepatitis B. Asian Pac J Cancer Prev 2015;16:5069-73.
18. Chanthra N, Payungporn S, **Chuaypen N**, Piratanantatavorn K, Pinjaroen N, Poovorawan Y, et al. Single nucleotide polymorphisms in STAT3 and STAT4 and risk of hepatocellular carcinoma in Thai patients with chronic hepatitis B. Asian Pac J Cancer Prev 2015;16:8405-10.
19. Chimparlee N, **Chuaypen N**, Khlaiphuengsin A, Pinjaroen N, Payungporn S, Poovorawan Y, et al. Diagnostic and prognostic roles of serum osteopontin and osteopontin promoter polymorphisms in hepatitis B-related hepatocellular carcinoma. Asian Pac J Cancer Prev 2015;16:7211-7.
20. Pratedrat P, Sopipong W, Makkoch J, Praianantathavorn K, **Chuaypen N**, Tangkijvanich P, et al. Single nucleotide polymorphisms in miR-149 (rs2292832) and miR-101-1 (rs7536540) are not associated with hepatocellular carcinoma in Thai patients with hepatitis B virus infection. Asian Pac J Cancer Prev 2015;16:6457-61.
21. **Chuaypen N**, Boonla C, Dissayabutra T, Predanon C, Ruangvejvorachai P, Waivijit U, Tosukhowong P. Increased intrarenal expression of sodium-dicarboxylate cotransporter-1 nephrolithiasis patients associates with acidic urine pH. Asian Biomedicine 2013; 7: 571-7.

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- 2007-2010 Bachelor degree of biochemistry, Department of biochemistry, Faculty of science, Kasetsart University
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WORK EXPERIENCE

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SCHOLARSHIPS

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- Chulalongkorn university alumni foundation scholarship
- Graduate thesis grant
- Doctoral Degree Chulalongkorn University 100th Year Birthday Anniversary

PRESENTATIONS AND CONFERENCES

- Proceedings of the 35th pharmacological and therapeutic society of Thailand meeting 2013 in a topic of "Human MiRNAs Targeting Hepatitis B Virus Genotype A-J by Computational Analysis"
- 52nd Annual scientific meeting 2013 in the theme of the meeting "Healthcare beyond boundaries: ASEAN Initiatives"
- Young scientist award from Asian Pacific Association for the Study of the Liver Single Topic Conference (APASL STC) 2017 in Nagasaki, Japan in a topic of "Association of TRAIL receptor 1 (TRAIL-R1) polymorphism with treatment response to pegylated interferon in chronic hepatitis B patients"

TECHNICAL SKILLS

- Experience working in molecular biology procedures such as PCR, real-time PCR, DNA/RNA/protein extraction, cloning, gel electrophoresis and cell culture.
- Experience working in blood sample such as separate serum, plasma or PBMC.

OTHER SKILLS

- Computer Skill :MS Office Program (Word,Excel, Power Point), Adobe photoshop
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PUBLICATIONS

- Poungpairoj P, Whongsiri P, Suwannasin S, Khlaiphuengsin A, Tangkijvanich P, Boonla C. Increased Oxidative Stress and RUNX3 Hypermethylation in Patients with Hepatitis B Virus-Associated Hepatocellular Carcinoma (HCC) and Induction of RUNX3 Hypermethylation by Reactive Oxygen Species in HCC Cells. Asian Pac J Cancer Prev. 2015;16(13):5343-8.

- Limothai U, Chuaypen N, Khlaiphuengsin A, Posuwan N, Wasitthankasem R, Poovorawan Y, et al. Association of interferon-gamma inducible protein 10 polymorphism with treatment response to pegylated interferon in HBeAg-positive chronic hepatitis B. Antivir Ther. 2016;21(2):97-106.

- Tangkijvanich P, Chittmittraprap S, Poovorawan K, Limothai U, Khlaiphuengsin A, Chuaypen N, et al. A randomized clinical trial of peginterferon alpha-2b with or without entecavir in patients with HBeAg-negative chronic hepatitis B: Role of host and viral factors associated with treatment response. J Viral Hepat. 2016;23(6):427-38.

- Khlaiphuengsin A, NP TT, Tangkijvanich P, Posuwan N, Makkoch J, Poovorawan Y, et al. Human miR-5193 Triggers Gene Silencing in Multiple Genotypes of Hepatitis B Virus. Microrna. 2015;4(2):123-30.

- Chimparlee N, Chuaypen N, Khlaiphuengsin A, Pinjaroen N, Payungporn S, Poovorawan Y, et al. Diagnostic and Prognostic Roles of Serum Osteopontin and Osteopontin Promoter Polymorphisms in Hepatitis B-related Hepatocellular Carcinoma. Asian Pac J Cancer Prev. 2015;16(16):7211-7.

- Khlaiphuengsin A, Kiatbumrung R, Payungporn S, Pinjaroen N, Tangkijvanich P. Association of PNPLA3 Polymorphism with Hepatocellular Carcinoma Development and Prognosis in Viral and Non-Viral Chronic Liver Diseases. Asian Pac J Cancer Prev. 2015;16(18):8377-82.

- Sriprapun M, Chuaypen N, Khlaiphuengsin A, Pinjaroen N, Payungporn S, Tangkijvanich P. Association of PINX1 but not TEP1 Polymorphisms with Progression to Hepatocellular Carcinoma in Thai Patients with Chronic Hepatitis B Virus Infection. Asian Pac J Cancer Prev. 2016;17(4):2019-25.

- Limothai U, Chuaypen N, Khlaiphuengsin A, Chittmittraprap S, Poovorawan Y, Tangkijvanich P. Association of vitamin-D-related genetic variations and treatment response to pegylated interferon in patients with chronic hepatitis B. Antivir Ther. 2017.

RESEARCH ARTICLE

Editorial Process: Submission:01/19/2018 Acceptance:07/23/2018

The Association of PNPLA3, COX-2 and DHCR7 Polymorphisms with Advanced Liver Fibrosis in Patients with HCV Mono-Infection and HCV/HIV Co-Infection

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Abstract

There is increasing evidence that host genetic variations may influence the natural history of chronic hepatitis C virus (HCV) infection. The aim of this study was to determine the association between single nucleotide polymorphisms (SNPs) of PNPLA3 (rs738409), COX-2 (rs689465) and DHCR7 (rs12785878) and advanced liver fibrosis in Thai patients. A total of 220 patients with HCV mono-infection, 200 patients with HCV/HIV co-infection and 200 healthy controls were enrolled. The SNPs were detected by allelic discrimination using real-time PCR with TaqMan probes. Liver stiffness measurement (LSM) was assessed by transient elastography. Our results showed that the distribution of the studied SNPs were not significantly different between the HCV mono- and co-infected groups. The frequencies AG and GG genotypes of rs689465 and GG genotype of rs12785878 were less commonly found in the HCV mono- and co-infected groups compare with healthy controls ($P<0.01$). Among patients with HCV infection, older age, HIV co-infection, GG genotype of rs738409 and GG genotype of rs689465 were independently associated with advanced liver fibrosis (LSM≥9.5 kPa) in multivariate analysis. Moreover, the percentage of patients with advanced liver fibrosis increased significantly along with the accumulated numbers of these risk genotypes. In conclusion, PNPLA3 (rs738409) and COX-2 (rs689465) polymorphisms were associated with advanced liver fibrosis in patients with HCV mono- and co-infection, suggesting that these variants might play an important role in progressive liver fibrosis in these patients.

Keywords: Polymorphisms- HCV- HIV- fibrosis- cirrhosi

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Introduction

Hepatitis C virus (HCV) infection is an important etiological factor of chronic liver disease worldwide, with an estimated of more than 170 million people currently infected with the virus (Lemoine and Thursz, 2014). Most infected individuals develop into chronic hepatitis, which could progress to cirrhosis and the occurrence of hepatocellular carcinoma (HCC) (Intaraprasong et al., 2016). Various viral, host and environmental factors have been influenced the chronicity and disease progression, including male sex, time of HCV infection, obesity, diabetes, alcohol consumption and co-infection with human immunodeficiency (HIV) (Hajarizadeh et al., 2013). In fact, HIV infection could modify the natural history of chronic HCV infection in co-infected patients, with a higher likelihood of fibrosis progression and cirrhosis. For example, our previous data showed that approximately 40% and 25% of HCV/HIV co-infected and HCV mono-infected patients had advanced liver fibrosis (Avihingsanon et al., 2014).

Host genetic variations have been implicated to influence the natural history of chronic HCV infection. Recent studies have shown that several single nucleotide polymorphisms (SNPs) are related to HCV infection susceptibility and disease progression (Matsuura and Tanaka, 2017). In this setting, Patatin-Like phospholipase domain containing protein 3 (PNPLA3) is involved in lipid storage and its activity has been reported in hydrolysis of triglyceride in the liver (Trepo et al., 2016). The genetic variations of PNPLA3 (rs738409) encoding isoleucine to methionine has been identified to be associated with liver steatosis and fi rosis in patients with HCV infection (Trepo et al., 2016). Moreover, several potent mediators of inflammation are thought to be involved in the process of persistent liver injury and progression of liver fibrosis. In this setting, the polymorphism of cyclooxygenase-2 (COX-2, rs689465) has been linked to pro-inflammatory metabolism, severity of liver fibrosi and HCC development (Miyashita et al., 2012; Bu and

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Zhao, 2013). In addition, there have increasing data that gene-associated with vitamin D metabolism plays an important role in severity of liver fibrosis (Grunhage et al., 2012). For example, a variant of 7-Dehydrocholesterol reductase (DHCR7) (rs12785878), the rate-limiting enzyme of vitamin D metabolism, is associated with lower vitamin D levels and promotes advance fibrosis in patients with chronic HCV infection (Petta et al., 2013). Previous data of our group have also suggested vitamin D defi iency was common in chronic HCV infection and might be associated with progressive liver fibrosis (Avihingsanon et al., 2014). Thus, the aim of this study was to determine the correlation of PNPLA3 (rs738409), COX-2 (rs689465) and DHCR7 (rs12785878) polymorphisms with severity of liver fibrosis in Thai individuals.

Materials and Methods

Patients

From 2011 to 2014, 220 patients with chronic HCV mono-infection and 200 patients with HCV/HIV co-infection were enrolled from King Chulalongkorn Memorial Hospital (Bangkok, Thailand) and the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT, Bangkok, Thailand), respectively. In addition, 200 healthy controls were recruited from The Thai Red Cross Society (Bangkok, Thailand) at the same period. HCV mono-infection was defined by anti-HCV and HCV RNA positive without evidence of HIV infection, while HCV/HIV co-infection was defined by anti-HCV, HCV RNA and anti-HIV positive. Patients with hepatitis B antigen surface antigen (HBsAg) positive or evidence of HCC were excluded.

All participants signed an informed consent before recruited into the study. The research protocol was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB 412/57 and 583/57).

Genotyping of the SNPs

Peripheral blood mononuclear cells (PBMCs) were stored at -80 ^{o}C until SNP genotyping was performed. Human genomic DNA was extracted and purified by phenol chloroform isoamyl alcohol method. Positive control was obtained from HeLa and HepG2 cell line.

PNPLA3 was performed with the forward primer 5´-TACCACGCCTCTGAAGGAAG-3´, the reverse primer 5´-CCCTGCTCACTTGGAGAAAG-3´ (Falleti et al., 2011) and COX-2 was obtained with the forward primer 5´- GAGCACTACCCATGATAGATGTTAAACA-3´, the reverse primer 5´ - TCTCGTTTTGGAACATAGT TGGATGAG-3´ (Miyashita et al., 2012). PCR amplifica ion was containing 0.5 µM forward and reverse primer, 0.5 µM deoxynucleotidetriphosphate (dNTP), 0.6 units of dreamTaq DNA polymerase (thermo scientific, USA) and 100-500 ng of DNA sample in total volume of 25 µl. PCR condition was consisted with denaturation at 95 ^{o}C for 30 s, annealing at 58°C for 30 s and elongation at 72 ^{o}C for 30 s in total 40 cycles. Genotyping was verified by TaqMan probe, PNPLA3 rs738409 (Assay ID: C_7241_10), COX-2 rs689465 (Assay ID: C_2517146_10) and DHCR7 rs12785878 (Assay ID: C_32063037_10) that classified genotype by allelic discrimination method (Applied Biosystems, Foster City, CA) (Zhang et al., 2012). PCR reaction for genotyping was performed with Perfect Taq Master Mix (5 PRIME, Darmstadt, Germany) in total volume of 10 µl as recommended by the manufacturer's instruction. The PCR conditions were followed by holding stage at 95°C for 10 min, denaturation at 92°C for 15 s and annealing/extension at 60°C for 1 min in total 40 cycles. The fluorescenc signals (VIC and FAM) that specific to each SNPs were detected and reported by the ABI Step One Plus real-time PCR system (Applied Biosystems).

Liver stiffness measurement

Liver stiffness measurement (LSM) was obtained from each patient with HCV mono-infection and HCV/HIV co-infection after fasting for at least 2 hours by transient elastography (FibroScan, Echosens, Paris, France). Results were recorded in kilopascals (kPa) as the median value of all measurements. The procedure was based on at least 10 validated measurements: the success rate (ratio between numbers of validated and total measurements) was over 60% and interquartile range was less than 30% (Castera et al., 2012). Liver fibrosis stages were defined according to LSM: F0-F1 (<7.1 kPa), F2 (7.1-9.4 kPa), F3 (9.5-14.0) and F4 (>14.0 kPa) (Avihingsanon et al., 2014).

Statistical analysis

Genotyping of the SNPs was reported in allelic discrimination plot. Data were presented as Mean ± standard deviation (SD) and categorical variables as frequency and percentage as appropriate. Frequencies (%) of genotype distribution in study population data were compared to the control groups using online GraphPad Software (http://www.graphpad.com). Moreover, the correlation of the studied SNPs with advanced liver fibrosis was calculated by using a binary variable and the univariate odds ratios (OR) in MedCal Software (http://www.medcalc.org). The results were considered as significant when P<0.05 (two-tailed) and frequencies by chi-square test using SPSS software version 22.0.

Results

Baseline characteristics of the participants

The characteristics of all participants are shown in Table 1. The mean age of the healthy control group was significantly higher compared with the other groups (P<0.001). However, there was no significant difference in mean age among the HCV mono-infected and HCV/HIV co-infected groups. HCV/HIV co-infected patients had higher proportion of male gender compared the other groups (P<0.001). In addition, HCV/HIV co-infected patients had significantly higher mean LSM compared with HCV mono-infected patients (P<0.001). There was no difference between HCV mono-infected and HCV/HIV co-infected patients in terms of serum alanine aminotransferase (ALT), HCV RNA levels and HCV genotype distribution.

PNPLA3 rs738409 genotype distribution

The genotype frequencies of PNPLA3 (rs738409) did not deviate from Hardy-Weinberg Equilibrium in all the studied participants. The genotype distributions and allele frequencies of the SNP are presented in Table 2. The frequencies of CC, CG and GG genotypes in HCV mono-infected patients were 53.2%, 40.3% and 6.5%, respectively, while the corresponding genotypes were 50.8%, 39.2%, and 10.0% in the HCV/HIV co-infected patients. Moreover, the corresponding genotype frequencies were 45.5%, 44.0% and 10.5% in the healthy controls. Compared with the healthy control group, HCV mono-infected patients had lower distribution of G allele [odds ratio (OR) =0.73, 95% confidence interval (CI) =0.55-0.99, P=0.043]. However, the genotype and allele frequencies were similar between controls and patients with HCV/HIV co-infection. In addition, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups.

COX-2 rs689465 genotype distribution

The genotype distributions and allele frequencies of COX-2 (rs689465) are shown in Table 2. In the HCV mono-infected group, the frequencies of AA, AG and GG genotypes were 78.2%, 19.5% and 2.3%, respectively. The corresponding genotypes was 76.0%, 21.0% and 3.0% in the HCV/HIV co-infection group, while their distribution in the healthy control group were 57.5%, 32.5% and 10.0%, respectively. Our results showed that the frequencies of AG and GG genotypes were less common in the HCV mono-infection group compare with healthy controls (OR=0.44, 95%CI=0.28-0.71; P<0.001 and OR=0.17, 95%CI=0.06-0.46; P<0.001, respectively). Similar results were found among HCV/HIV co-infected patients compared with the healthy controls regarding the distribution of AG and GG genotypes (OR=0.49, 95%CI=0.31-0.77; P=0.002 and OR= 0.23, 95%CI=0.09-0.58; P=0.002, respectively). However, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups (Table 2).

DHCR7 rs12785878 genotype distribution

The frequency of TT, TG and GG genotypes of DHCR7 (rs12785878) in each group are shown in Table 2. In the HCV mono-infected group, their frequencies were 56.8%, 38.2% and 5.0%, respectively, while their distribution in the HCV/HIV co-infected group were 67.0%, 39.5% and 3.5%, respectively. The corresponding genotypes in the healthy control group were 46.0%, 42.0% and 12.0%, respectively. The GG genotype was less frequently distributed in the HCV mono-infected group than in the healthy control group (OR=0.34, 95% CI=0.16-0.72, P=0.005). Likewise, GG genotype was less common in the HCV/HIV co-infected group than the healthy control group (OR=0.24, 95%CI=0.10-0.57, P=0.0001). However, the genotype distributions and allele frequencies were not different between the HCV mono-infected and HCV/HIV co-infected groups (Table 2).

Independent and additive effects of the SNPs associated with advanced liver fibrosis

To identify factors associated advanced liver fibrosis (F3 and F4, LSM≥9.5 kPa), baseline characteristics including patient's age, gender, ALT, HIV co-infection, HCV RNA viral load, HCV genotype, SNPs rs738409, rs689465 and rs12785878 were evaluated by logistic regression analyses. The data showed that age, HIV co-infection, SNPs rs738409 and rs689465 were associated with advanced liver fibrosis in univariate and multivariate analyses (Table 3).

The combined effect of the risk genotypes, including

Table 1. Baseline Characteristics of the Participants in This Study

	HCV mono-infection (n=220)	HCV/HIV co-infection (n= 200)	Healthy control (n=200)	P
Age (years)	43.1±10.4	42.6±7.2	47.5±5.2	<0.001
Gender				
Male (%)	155 (70.5%)	179 (89.5%)	111 (55.5%)	<0.001
Female (%)	65 (29.5%)	21 (10.5%)	89 (44.5%)	
ALT (IU/L)	71.5± 57.4	75.4± 61.3	ND	0.872
HCV-RNA (log_{10} IU/ml)	6.2± 2.1	6.3± 1.9	ND	0.554
HCV genotypes			ND	0.372
1	75 (34.1%)	76 (38.0%)		
3	105 (47.7%)	80 (40.0%)		
6	40 (18.2%)	30 (15.0%)		
Unknown	0(0%)	14 (7.0%)		
Fibrosis stage			ND	<0.001
F0-F1/F2	150 (68.2%)	97 (48.5%)		
F3/F4	70 (31.8%)	103 (51.5%)		
Liver stiffness (kPa)	10.2±8.6	13.3±10.5	ND	0.001

HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; ND, No data; Fibrosis stage cutoff; F0-F1 (<7.1 kPa), F2 (7.1-9.4 kPa), F3 (9.5-14.0) and F4 (>14.0 kPa).

Table 2. Genotype and Allele Frequencies of the Studied SNPs in Patients with HCV Infection and Controls

SNPs	HCV (n=220)	HCV-HIV (n=200)	Control (n=200)	HCV vs. control		HCV-HIV vs. control		HCV-HIV vs. HCV	
				OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
PNPLA3									
Allelic model									
Major (C)	325	282	270	1		1		1	
Minor (G)	115	118	130	0.73 (0.55-0.99)	0.043*	0.87 (0.64-1.17)	0.359	1.18 (0.87-1.60)	0.277
Additive model									
CC	120	102	91	1		1		1	
CG	85	78	88	0.73 (0.49-1.10)	0.131	0.79 (0.52-1.20)	0.268	1.08 (0.72-1.62)	0.711
GG	15	20	21	0.54 (0.26-1.11)	0.094	0.85 (0.43-1.67)	0.636	1.57 (0.76-3.22)	0.22
Dominant model									
CC	120	102	91	1		1		1	
CG+GG	100	98	109	0.70 (0.47-1.02)	0.064	0.80 (0.54-1.19)	0.271	1.15 (0.79-1.69)	0.467
Recessive model									
CC+CG	205	180	179	1		1		1	
GG	15	20	21	0.62 (0.31-1.25)	0.181	0.95 (0.50-1.81)	0.869	1.52 (0.755-3.05)	0.241
COX-2									
Allelic model									
Major (A)	387	346	295	1		1		1	
Minor (G)	53	54	105	0.38 (0.27-0.55)	<0.001*	0.44 (0.31-0.63)	<0.001*	1.14 (0.76-1.71)	0.528
Additive model									
AA	172	152	115	1		1		1	
AG	43	42	65	0.44 (0.28-0.70)	<0.001*	0.49 (0.31-0.77)	0.002*	1.11 (0.69-1.78)	0.682
GG	5	6	20	0.17 (0.06-0.46)	<0.001*	0.23 (0.09-0.58)	0.002*	1.36 (0.41-4.54)	0.619
Dominant model									
AA	172	152	115	1		1		1	
AG+GG	48	48	85	0.38 (0.25-0.58)	<0.001*	0.43 (0.28-0.66)	<0.001*	1.13 (0.72-1.78)	0.595
Recessive model									
AA+AG	215	194	180	1		1		1	
GG	5	6	20	0.21 (0.08-0.57)	0.002*	0.28 (0.11-0.71)	0.007*	1.33 (0.40-4.43)	0.642
DHCR7									
Allelic model									
Major (T)	334	307	268	1		1		1	
Minor (G)	106	93	132	0.64 (0.48-0.87)	0.004*	0.62 (0.45-0.84)	0.002*	0.96 (0.69-1.31)	0.774
Additive model									
TT	125	114	92	1		1		1	
TG	84	79	84	0.74 (0.49-1.10)	0.138	0.76 (0.50-1.15)	0.19	1.03 (0.69-1.54)	0.88
GG	11	7	24	0.34 (0.16-0.72)	0.005*	0.24 (0.10-0.57)	0.001*	0.70 (0.26-1.86)	0.472
Dominant model									
TT	125	114	92	1		1		1	
TG+GG	95	86	108	0.65 (0.44-0.95)	0.027*	0.64 (0.43-0.95)	0.028*	0.99 (0.67-1.46)	0.97
Recessive model									
TT+TG	209	193	176	1		1		1	
GG	11	7	24	0.39 (0.18-0.81)	0.012*	0.27 (0.11-0.63)	0.003*	0.69 (0.26-1.81)	0.451

OR, odd ratio; CI, confidence interva

rs738409 (CG+GG) and rs689465 AG+GG) on the presence of advanced liver fibrosis were further investigated. Among patients with advanced liver fib osis, there were 66 (39.1%) patients who did not carry any risk genotype, while there were 67 (37.6%), 31 (53.4%) and 9 (60.0%) patients who had 1, 2 and 3 risk genotypes, respectively (P=0.044, Chi-square test for trend analysis). These data showed that the percentage of patients with advanced liver fibrosis increased significantly along with the accumulated numbers of the risk genotypes.

Table 3. Univariate and Multivariate Regression Analyses of Factors Associated with Advanced Liver Fibrosis (LSM ≥ 9.5 kPa)

Factors	Category	Univariate analysis		Multivariate analysis	
		Odd ratio (95%CI)	P	Odd ratio (95%CI)	P
Baseline					
Age (years)	< 40 vs. ≥ 40	4.08 (2.65-6.27)	<0.001*	4.44 (2.82-6.98)	<0.001*
Sex	Male vs. Female	1.59 (0.96-2.62)	0.07		
ALT (U/L)	< 75 vs. ≥ 75	0.98 (0.42-2.30)	0.965		
HIV co-infection	yes vs. no	2.28 (1.53-3.38)	<0.001*	2.39 (1.56-3.67)	<0.001*
Log10 HCV RNA (IU/mL)	< 6.0 vs. ≥ 6.0	1.10 (0.57-2.15)	0.81		
HCV genotypes	1 vs. 3 and 6	1.01 (0.82-1.24)	0.92		
SNPs					
PNPLA3 (rs738409)	GG vs. non-GG	1.42 (1.01-2.02)	0.049*	1.48 (1.01-2.16)	0.045*
COX-2 (rs689465)	GG vs. non-GG	2.59 (1.20-5.61)	0.016*	2.63 (1.19-5.80)	0.017*
DHCR7 (rs12785878)	GG vs. non-GG	0.84 (0.51-1.38)	0.491		

ALT, alanine aminotransferase; SNPs, Single nucleotide polymorphisms; CI, confidence interva

Discussion

Natural history and clinical outcome of HCV infection display remarkably inter-individual differences. Previous data have shown that more than 70% of acute HCV infection progress to chronic infection, in which approximately 20% of cases will develop towards cirrhosis and finally HCC (Lemoine and Thursz, 2014). Increasing data have indicated that the clinical course and disease progression of HCV infection is related to interaction of various factors including viral, host and environmental factors (Matsuura and Tanaka, 2017). Regarding host genetic variations, previous genome-wide association studies (GWAS) have shown that SNPs near the interferon lambda-3 (IFNL3) and interferon lambda-4 (IFNL4) genes are predictors of spontaneous HCV clearance and response to pegylated interferon-based therapy (Tanaka et al., 2009; Prokunina-Olsson et al., 2013). Recent GWAS have also identifi d additional genetic variations associated with the severity of liver fibrosis and HCC development (Matsuura et al., 2017).

In this report, multivariate analysis showed that PNPLA3 GG genotype was an independent risk factor associated with advanced liver fib osis assessed by LSM. Indeed, LSM with transient elastography appears to be a reliable non-invasive tool to detect significant fibrosis or cirrhosis in patients with chronic HCV infection (Houot et al., 2016). Our results are in agreement with previous reports demonstrating that PNPLA3 polymorphism influenced the progression of liver fibrosis in HCV mono-infected and HCV/HIV co-infected patients (Trepo et al., 2011; Ali et al., 2016; Jimenez-Sousa et al., 2016; Nunez-Torres et al., 2016). For instance, an analysis of data from participants in a large cohort of HCV mono-infection showed that PNPLA3 genotype was significantly associated with the presence of cirrhosis after adjusting for other factors (Ali et al., 2016). In addition, a recent study reported that the presence of PNPLA3 (rs738409) G allele increased the odds of having advanced liver fibrosis in HCV/HIV co-infected patients (Jimenez-Sousa et al., 2016). In contrast, an association between the PNPLA3 polymorphism and the degree of liver fibrosis was not confirmed in other reports of HCV mono- and co-infection (Nakamura et al., 2013; Sagnelli et al., 2016).

COX-2 represents an inducible enzyme that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation involved in several cellular processes including proliferation, carcinogenesis and metastasis (Simmons et al., 2004). Our data showed that COX-2 (rs689465) GG genotype was identified as a predictor of advanced liver fibrosis by multivariate analysis. This result was in accordance with a Japanese study demonstrating that rs689465 polymorphism was linked to liver disease progression in patients with HCV mono-infection (Miyashita et al., 2012). Moreover, a recent meta-analysis have suggested that COX-2 (rs689465) variant might be a factor associated with HCC risk in Asian populations (Bu and Zhao, 2013). Interestingly, our data also show for the first that the combined testing of PNPLA3 (rs738409) and COX-2 (rs689465) could exhibit an additive effect towards the presence of advanced liver fibrosis. Taken together, these results indicate that PNPLA3 and COX-2 variants might play a significant role in the progression of liver fibrosis in HCV mono-infected and HCV/HIV co-infected patients.

The mechanism underlying this association remains unclear as PNPLA3 does not seem to have a direct impact on the natural history of chronic HCV infection. In fact, rs738409 GG allele might not directly affect the PNPLA3 mRNA levels but it could result in an inhibition of PNPLA3 function and influence towards susceptibility to intrahepatic fat accumulation. As a result, it could be explained that PNPLA3 variant promotes liver fat accumulation, which in turn leads to progressive steatosis and, ultimately, the development of liver fibrosis and cirrhosis (Trepo et al., 2011). Regarding the role of COX-2, previous data suggested that patients with risk allele of rs689465 had higher expression levels of COX-2 mRNA in the liver and lymphoid cells (Miyashita et al., 2012). It was also showed that COX-2 over-expression in the hepatocytes was associated with advanced liver

fibrosis in patients with chronic HCV infection (Nunez et al., 2004). Thus, it is speculated that COX-2 GG genotype might promote higher levels of COX-2 expression, resulting greater liver inflammation and progressive liver fibrosis

It has been recently showed that vitamin D, a potent immune-modulator, is associated with the pathogenesis of various disorders, including chronic HCV infection (Grunhage et al., 2012). In fact, hypovitaminosis D is frequently found among patients with chronic HCV infection. In our previous report, vitamin D insufficiency and deficiency were found in more than 60% of patients with HCV mono- and co-infection (Avihingsanon et al., 2014). In addition, common SNPs in vitamin D-related genes, such as DHCR7, CYP27B1, vitamin D receptor (VDR) and vitamin D binding protein (DBP), are linked to clinical manifestation and treatment response in patients with chronic HCV infection (Grunhage et al., 2012). For instance, a recent Italian report found an association between severity of liver fibrosis and DHCR7 variant in patients with HCV genotype 1 infection (Petta et al., 2013). In contrast, our cohort did not show that DHCR7 genotypes infl enced progressive liver fi rosis in patients with chronic HCV infection. This discrepancy between studies might be related to the heterogeneity of reports in terms of studied population, genetic background, sample size, HCV genotypes, and differences in methods of liver fibrosis assessment. In light of this inconsistency, the roles of vitamin D-related SNPs in the clinical outcome of chronic HCV infection need to be elucidated in further studies.

In conclusion, PNPLA3 (rs738409) and COX-2 (rs689465) polymorphisms were associated with advanced liver fibrosis, suggesting that these variants might play an important role in liver fibrogenesis in patients with HCV mono- and co-infection. Given a higher chance for developing advanced fibrosis/cirrhosis and subsequent complications, patients harboring these risk genotypes may warrant closely monitored the clinical progression and being prioritized for antiviral therapy. Further cohorts with larger sample sizes should be performed to confirm these observations.

Conflict of Interest

The authors declare no conflicts of interest

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ORIGINAL ARTICLE

Plasma B-cell activating factor levels and polymorphisms in hepatitis B-related hepatocellular carcinoma: Clinical correlation and prognosis

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Abstract

Background: B-cell activating factor (BAFF), an important cytokine for B lymphocyte activation, has been shown to be increased in chronic hepatitis B virus (HBV) infection.

Objectives: This study aimed at evaluating clinical correlation and prognostic role of plasma BAFF and related polymorphisms in patients with HBV-related hepatocellular carcinoma (HCC).

Methods: Plasma BAFF levels were measured from 100 healthy controls and 490 patients with chronic HBV infection (200 with HCC and 290 without HCC). The rs9514828 and rs12583006 polymorphisms were determined by allelic discrimination.

Results: The HCC group had significantly higher BAFF levels compared with the non-HCC group and healthy controls. Among the non-HCC group, the HBeAg-positive subgroup had higher BAFF levels compared with the HBeAg-negative subgroup. In the HCC group, high BAFF levels at initial presentation significantly correlated with alpha-fetoprotein levels, Child-Pugh classification, tumor size and BCLC stage. Multivariate analyses showed that elevated BAFF concentration (≥ 1,100 pg/ml) was a significant and independent prognostic factor of overall survival in patients with HCC (OR = 2.28, 95%CI: 1.07-4.87; $P = 0.034$). HCC patients with high BAFF levels (≥ 1,100 pg/ml) had a poorer median survival than those with low levels ($P < 0.001$, log-rank test). Regarding BAFF polymorphisms, the frequency of rs9514828 CT + TT genotypes was higher distributed in patients with chronic HBV infection compared with healthy controls (58.0% vs. 46.0%, $P = 0.029$).

Conclusions: Our data demonstrate for the first time that elevated plasma BAFF levels at baseline exhibit clinical correlation in terms of disease severity and overall survival in HCC patients. Thus, plasma BAFF at initial diagnosis could serve as a prognostic marker for HBV-related HCC.

Key words: BAFF, B cells, HBV, HCC, polymorphisms

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Introduction

Hepatitis B virus (HBV) infection is an essential public health problem worldwide. Individuals chronically infected with HBV have a diverse clinical manifestation including chronic hepatitis, progressive fibrosis, cirrhosis, and finally hepatocellular carcinoma (HCC) development.[1] Accumulative recent evidence has suggested that the pathogenesis and consequence of HBV infection is greatly linked to immune-mediated host -virus interactions.[2,3] During acute infection, vigorous and specific B and T-cell activities are coordinately involved in viral clearance and self-limited hepatitis. By contrast, the immune responses display functional impairment in individuals developing chronic HBV infection.[2] Currently, it is documented that

injury and eventually leads to the diversity of clinical outcome of HBV infection.[4] Unlike the importance of T-cell mediated immune response, data regarding the role of B-cell immunity in the pathogenesis and prognosis of chronic HBV infection are less well documented.

B cells exhibit an essential role in humoral immunity by producing antibodies, modulating inflammatory cytokines and inducing the T-cell response.[5,6] In general, peripheral B lymphocytes require B cell-activating factor (BAFF) for their activation, differentiation and survival. BAFF is a member of the tumor necrosis factor superfamily (TNFSF13B) and an IFN stimulated gene, which is produced by several cells such as neutrophils, monocytes, macrophages, dendritic cells and activated T cells.[7] Previous reports have demonstrated that circulating BAFF levels are highly expressed and correlated with clinical activity and outcome of several autoimmune diseases and hematological malignancies.[8-10] Apart from its established role in these diseases, enhanced BAFF levels were also observed in the context of many infections and non-hematological malignancies, suggesting it may play a pathogenic role in diverse disorders[11] For instance, enhanced BAFF expression was detected in hepatitis C virus (HCV) infection, particularly among patients with mixed cryoglobulinemia.[12,13] Moreover, it was recently shown that increased BAFF levels appeared to correlate with disease severity in patients with chronic HBV infection and was independently associated with the occurrence of HCC.[14] These data suggest that BAFF may contribute to progressive liver disease and HCC development in patients with chronic HBV infection. However, its correlation with clinical characteristics and prognosis of HCC has not yet been completely evaluated.

Regarding host genetic variation, single nucleotide polymorphisms (SNPs) of the *BAFF* gene, including rs9514828 and rs12583006, have been shown to be associated with alteration of BAFF expression and linked to several autoimmune and hematological disorders.[15-17] As available data in chronic HBV infection are limited,[18] it is unclear whether these SNPs might be associated with clinical severity and prognosis of patients with HCC. To address these issues, we determined whether plasma BAFF and these polymorphisms were associated with clinical characteristics and outcome in patients with HBV-related HCC.

Methods

Patients

Stored samples for the measurement of plasma BAFF levels and polymorphisms were obtained from patients who were diagnosed of HBV-related HCC for the first time at King Chulalongkorn Memorial Hospital, Bangkok, Thailand between May 2010 and December 2015. The diagnosis of HBV infection was confirmed by the presence of serum hepatitis B s antigen (HBsAg). HCC was diagnosed on the basis of typical imaging studies and/or histopathology (fine needle aspiration, core liver biopsy or surgical resection) according to the standard guideline.[19] Diagnostic criteria of HCC by imaging studies were based on findings of focal hepatic lesions with hyperattenuation at the arterial phase, hypoattenuation at the portal phase in dynamic CT or MRI. The clinical parameters of patients with HCC at initial diagnosis were collected, which included sex, age, liver function tests, Child-Pugh classification, serum alpha-fetoprotein (AFP) level and HCC staging classified by the Barcelona Clinic Liver Cancer (BCLC) system.[20]

Patients with chronic HBV infection, who had no evidence of HCC were recruited as the non-HCC group. These patients attended King Chulalongkorn Memorial Hospital and had been followed up every 4-6 months during the same period as patients with HCC. Chronic HBV infection was diagnosed by the positivity of serum HBsAg at least 6 months. Exclusions criteria for this group were as follows: (1) co-infection with HCV and/or human immunodeficiency virus (HIV); (2) evidence of other malignancies or autoimmune disorders during follow-up. Patients with chronic HBV infection were classified into inactive carrier (IC) and immune active (IA) phased based on the criteria of the American Association for the Study of Liver Diseases (AASLD).[21] Moreover, healthy individuals recruited from blood donors at National Blood Centre Thai Red Cross Society, Bangkok, Thailand were used as the healthy control group.

The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB no. 438/60) and participants had provided written informed consent. The study followed the Helsinki Declaration and Good Clinical Practice guidelines.

Plasma BAFF levels and HBV marker assays

Baseline plasma BAFF levels were determined by ELISA (R&D Systems) according to the manufacturer's protocol. Qualitative measurements of HBsAg and HBeAg were tested by commercial available enzyme-linked immunosorbent assays (Abbott Laboratories, Chicago, IL). HBV DNA levels were tested by Abbott Real Time HBV assay (Abbott Laboratories).

Genotyping of BAFF polymorphism

Genomic DNA was extracted from 100 µl of peripheral blood mononuclear cells (PBMCs) by the phenol-chloroform isolation method according to the standard method. The quality of DNA was then measured using spectrophotometer (NanoDrop 2000c, Thermo Scientific). Genotyping of rs9514 828 was performed by using polymerase chain reaction (PCR) with restriction fragment length polymorphism analysis. PCR was performed by using PCR master mix (Thermo scientific) and primers were 5'-GGCACAGTCAACATGGGAGT-3' (forward) and 5'-GCTAAGTGTTTTAGCATTGAATTG-3' (reverse) according to the previous study.[16] A thermal condition was initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C 30 sec, 58°C for 30 sec and 72°C for 1 min and a final extension at 72°C for 7 min. The PCR product was digested by BSrBI (New England Biolabs) and followed by 2% agarose gel electrophoresis. Another BAFF genotyping rs12583006 was performed with real-time PCR based on TaqMan genotyping assay (C_11705495_10, Applied Biosystem). The reaction was performed by using TaqMan genotyping master mix (Applied Biosystems) and 20X primers and probes mixture (TaqMan SNP Genotyping Assay, Applied Biosystems). The real-time PCR condition was performed in ABI 7500 Real Time PCR System (Applied Biosystems) according to the manufacturer's protocol. A thermal condition was as follow, 10 min at 95°C was hold for an initial denaturation, then followed

by 40 cycles of amplification including denaturation at 95°C for 15 sec, and annealing/extension at 60°C for 1 min. Positive and negative controls were included in each experiment in order to confirm the results.

Statistical analysis

Statistical analysis was performed with SPSS statistics version 22 (SPSS Inc., Chicago, IL) and GraphPad Prism v5.0 (GraphPad Software, San Diego, CA). Values are presented as mean ± standard deviation (SD), and percentages as appropriate. Comparisons between groups were assessed by the $\chi2$, Student's t-test or Bonferroni correction method for quantitative variables. Correlations between parameters were analyzed by the Pearson correlation. Survival curves in patients with HCC were established using the Kaplan-Meier method and differences between curves were assessed by the log-rank test. The Cox regression analysis was performed to identify independent factors associated with overall survival (OS) of patients with HCC. P values < 0.05 were indicated statistical significance.

Results

Clinical characteristics

Table 1 compares baseline characteristics of all subjects enrolled in this study. Patients with HCC were older and had male gender distribution than patients without HCC and healthy controls ($P < 0.001$). Compared with the non-HCC group, patients with HCC had higher mean aspartate aminotransferase (AST), total bilirubin (TB), serum albumin, platelet counts, and AFP levels. In addition, patients with HCC had higher fibrosis-4 (FIB-4) index, a non-invasive scoring system for assessing liver fibrosis, and a higher frequency of cirrhosis than the non-HCC group. However, there was no difference between groups in terms of alanine aminotransferase (ALT), HBV DNA level and HBeAg positivity.

Comparison of plasma BAFF levels between studied groups

Plasma BAFF levels in patients with HCC obtained at the time of diagnosis ranged from 288.8 to 79.8 pg/ml, with a mean of 1330.7 ± 793.2 pg/ml. The average level of plasma BAFF levels in this group was significantly higher than that of the non-HCC group (906.5 ± 275.6 pg/ml; ranged from 476.0 to 3410.0 pg/ml) and healthy controls (845.7 ± 158.1 pg/ml; ranged from 487.5 to 1165.7 pg/ml, $P < 0.001$). Plasma BAFF level in the non-HCC group was also higher than in the healthy controls ($P = 0.037$) (**Figure 1A**).

Plasma BAFF levels in subgroups of patients without HCC

Among the non-HCC group, patients whose clinical feature categorized in the IA phase (n = 190) had significantly higher mean BAFF level than those classified in the IC phase (n = 100) (930.5 ± 315.3 pg/ml vs. 860.9 ± 169.4 pg/ml, $P = 0.015$). If categorized patients based on HBeAg status, patients with HBeAg positivity (n = 95) had significantly higher mean BAFF level than those with HBeAg negativity (n = 195) (991.0 ± 401.4 pg/ml vs. 865.4 ± 172.9 pg/ml, $P = 0.004$). Likewise, patients with cirrhosis (n = 52) exhibited higher average BAFF level than those without cirrhosis (n = 238) (1047.6 ± 440.0 pg/ml vs. 875.7 ± 213.6 pg/ml, $P = 0.008$) (**Figure 1B**).

In the non-HCC group, plasma BAFF levels were positively correlated with AST (r = 0.371, $P < 0.001$), ALT (r = 0.435, $P < 0.001$), HBV DNA (r = 0.140, $P = 0.021$), AFP (r = 0.481, $P < 0.001$) and FIB-4 index (r = 0.362, $P < 0.001$). There was no correlation between plasma BAFF levels and other clinical parameters (age, sex, total bilirubin, platelet counts and serum albumin).

Plasma BAFF levels and clinical features in patients with HCC

To evaluate the association between plasma BAFF levels and clinical features, the patients with HCC were divided into two

Table 1. Baseline characteristics of all subjects in the study

Baseline Characteristics	Healthy controls (n = 100)	Patients without HCC (n = 290)	Patients with HCC (n = 200)	*P*
Age (years)	49.3 ± 5.2	42.9 ± 11.8	58.1 ± 11.9	< 0.001*
Gender (Male)	65 (65.0)	174 (60.0)	168 (84.0)	< 0.001*
Aspartate aminotransferase (IU/L)		39.6 ± 35.9	95.6 ± 102.2	< 0.001*
Alanine aminotransferase (IU/L)		58.9 ± 70.3	59.5 ± 54.3	0.915
Serum albumin (g/dL)		4.4 ± 0.4	3.6 ± 0.6	< 0.001*
Total bilirubin (mg/dL)		0.7 ± 0.3	1.2 ± 0.7	< 0.001*
Platelet count (10^9/L)		228.6 ± 54.4	200.0 ± 126.9	0.003*
HBeAg positivity		95 (33.0)	58 (29.0)	0.468
Log10 HBV DNA (IU/mL)		4.8 ± 2.2	4.5 ± 1.5	0.199
Alpha fetoprotein (ng/mL)		5.3 ± 14.5	17203.5 ± 60745.5	0.007*
FIB-4 index		1.26 ± 0.83	4.87 ± 4.14	< 0.001*
Presence of cirrhosis		52 (17.9)	168 (84.0)	< 0.001*
BCLC stage (0-A/B/C-D)		-	61(30.5)/76(38.0)/3(31.5)	-

Data expressed as mean ± SD or n (%) as appropriate; *, *P*-value < 0.05

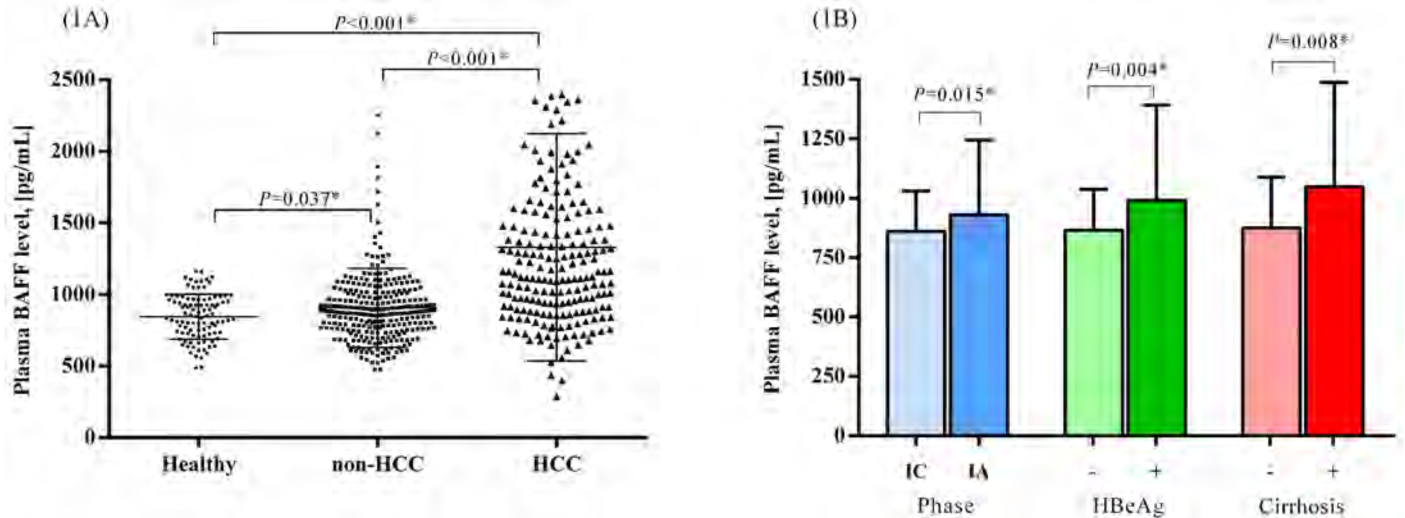

Figure 1. Plasma BAFF levels (A) Each group of patients and healthy controls (B) Subgroups of patients without HCC

groups based on their median value (approximately 1100 pg/ml) in all HCC patients. Accordingly, there were 99 and 101 patients with low and high levels of plasma BAFF, respectively. The correlations of low and high BAFF levels and various clinical parameters are summarized in **Table 2**. It was clearly shown that high BAFF levels were significantly correlated with serum AFP, severity of liver disease determined by Child-Pugh Classification and advanced BCLC stage. However, there was no correlation between plasma BAFF level and patient age, gender, platelet count, HBV DNA level and FIB-4 index.

Distribution of BAFF polymorphisms

Prevalence of the SNPs in the BAFF gene including rs9514828 and rs12583006 in each group of subjects are summarized in **Table 3**. There was no difference in the prevalence of rs9514828 genotypes between patients with HCC and non-HCC, as well as between patients with HCC and healthy controls. However, patients with chronic HBV infection (including HCC and non-HCC) had a significantly higher prevalence of CT and CT + TT compared with healthy controls. Regarding rs12583006 genotypes, there was no difference in their

Table 2. Relationship between plasma BAFF levels and characteristics of patients with HCC

Variables	Low BAFF (< 1100 pg/ml) (n = 99)	High BAFF (≥ 1100 pg/ml) (n = 101)	*P*
Age (years)	58.2 ± 11.9	58.0 ± 11.9	0.900
Gender			0.177
Male (n = 168)	87 (87.9)	81 (80.2)	
Female (n = 32)	12 (12.1)	20 (19.8)	
Aspartate aminotransferase (IU/L)	72.4 ± 71.8	118.4 ± 121.1	0.001*
Alanine aminotransferase (IU/L)	59.3 ± 17.5	59.8 ± 51.2	0.954
Serum albumin (g/dL)	3.8 ± 0.6	3.4 ± 0.5	< 0.001*
Total bilirubin (mg/dL)	1.0 ± 0.6	1.3 ± 0.8	0.008*
Platelet count (10^9/L)	188.8 ± 121.7	210.9 ± 131.5	0.221
Log10 HBV DNA (IU/mL)	4.5 ± 1.5	4.4 ± 1.5	0.879
Alpha fetoprotein (ng/mL)	5210.3 ± 16801.2	28735.4 ± 8208.0	0.016*
FIB-4 index	4.26 ± 3.92	5.46 ± 4.28	0.069
Child-Puge class			0.027*
A (n = 158)	89 (87.3)	69 (70.4)	
B or C (n = 42)	13 (12.7)	29 (29.6)	
BCLC tumor stage			< 0.001*
0-A (n = 61)	40 (40.4)	21 (20.8)	
B (n = 76)	41 (41.4)	35 (34.7)	
C-D (n = 63)	18 (18.2)	45 (44.6)	

Data expressed as mean ± SD or n (%) as appropriate; *, *P*-value < 0.05

Table 3. Prevalence of polymorphisms in studied groups

Polymorphisms	Healthy controls (n = 100)	Patients without HCC (n = 290)	Patients with HCC (n = 200)	Patients with and without HCC (n = 490)	HCC vs. Healthy controls		HCC vs. Non-HCC		Non-HCC and HCC vs. Healthy controls	
					OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs9514828										
Genotype frequency										
CC	54 (54.0)	116 (40.0)	90 (45.0)	206 (42.0)	1.00		1.00		1.00	
CT	36 (36.0)	142 (49.0)	90 (45.0)	232 (47.4)	1.50 (0.90-2.51)	0.121	0.82 (0.56-1.20)	0.299	1.69 (1.06-2.68)	0.026*
TT	10 (10.0)	32 (11.0)	20 (10.0)	52 (10.6)	1.20 (0.52-2.75)	0.667	0.81 (0.43-1.50)	0.496	1.36 (0.65-2.86)	0.412
CT + TT	46 (46.0)	174 (60.0)	110 (55.0)	284 (58.0)	1.43 (0.89-2.32)	0.142	0.81 (0.57-1.17)	0.271	1.62 (1.05-2.49)	0.029*
Allele frequency										
C	144 (72.0)	374 (64.5)	270 (67.5)	644 (65.7)	1.00		1.00		1.00	
T	56 (28.0)	206 (35.5)	130 (32.5)	336 (34.3)	1.24 (0.85-1.80)	0.261	0.87 (0.67–1.14)	0.328	1.34 (0.96-1.88)	0.086
rs12583006										
Genotype frequency										
AA	19 (19.0)	55 (19.0)	36 (18.0)	91 (18.6)	1.00		1.00		1.00	
AT	42 (42.0)	147 (50.7)	87 (43.5)	234 (47.8)	1.09 (0.56-2.13)	0.793	0.90 (0.55-1.49)	0.691	1.16 (0.64-2.11)	0.618
TT	39 (39.0)	88 (30.3)	77 (38.5)	165 (33.6)	1.04 (0.53-2.05)	0.905	1.34 (0.80-2.25)	0.274	0.88 (0.48-1.62)	0.688
AT + TT	81 (81.0)	235 (81.0)	164 (82.0)	399 (81.4)	1.07 (0.58-1.98)	0.833	1.07 (0.67-1.70)	0.787	1.03 (0.59-1.78)	0.920
Allele frequency										
A	80 (40.0)	257 (44.3)	159 (39.8)	416 (42.4)	1.00		1.00		1.00	
T	120 (60.0)	323 (55.7)	241 (60.2)	564 (57.6)	1.01 (0.71-1.43)	0.953	1.21 (0.93-1.56)	0.156	0.90 (0.66-1.23)	0.523

Data expressed as n (%); OR = Odd ratio; CI = confidence intervals; *, *P*-value < 0.05

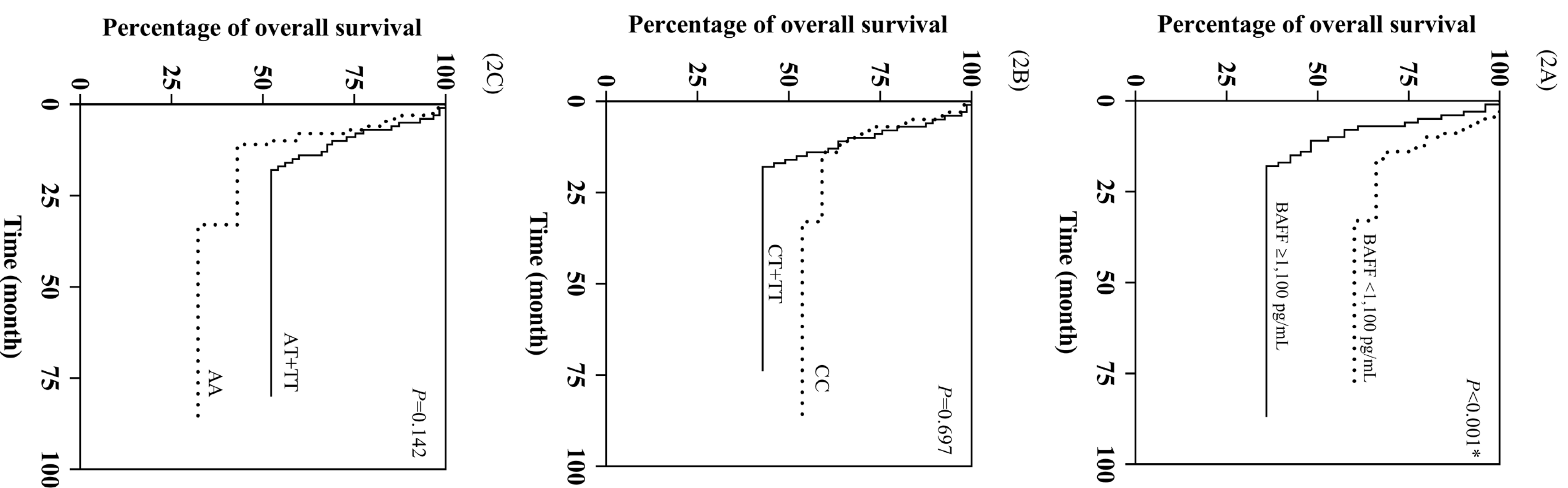

Figure 2. Overall survivals of patients with HCC regarding to BAFF levels and polymorphisms (A) plasma BAFF levels (B) rs9514828 genotypes (C) rs12583006 genotypes

Table 4. Factors associated with overall survival in patients with HCC

Factors	Category	Overall survival			
		Univariate analysis		Multivariate analysis	
		OR (95%CI)	*P*	OR (95%CI)	*P*
Age (years)	< 60 vs. ≥ 60	1.98 (1.14-3.45)	0.016*	0.69 (0.30-1.60)	0.387
Gender	Male vs. Female	1.35 (0.70-2.61)	0.374		
Aspartate aminotransferase (IU/L)	< 60 vs. ≥ 60	3.14 (1.76-5.63)	< 0.001*	0.92 (0.39-2.19)	0.853
Alanine aminotransferase (IU/L)	< 60 vs. ≥ 60	2.74 (1.62-4.65)	< 0.001*	1.34 (0.63-2.82)	0.445
Platelet count (10^9/L)	≥ 150 vs. < 150	2.94 (1.55-5.57)	0.001*	1.94 (0.75-5.02)	0.174
Log10 HBV DNA (IU/mL)	< 4.0 vs. ≥ 4.0	0.78 (0.39-1.56)	0.475		
Child-Pugh classification	A vs. B and C	1.42 (0.67-3.02)	0.361		
Alpha fetoprotein (ng/mL)	< 100 vs. ≥ 100	5.91 (2.99-11.68)	< 0.001*	3.64 (1.53-8.64)	0.003*
FIB-4 index	< 3.40 vs. ≥ 3.40	0.87 (0.52-1.51)	0.656		
Tumor size (cm.)	< 5.0 vs. ≥ 5.0	10.55 (4.69-23.75)	< 0.001*	2.10 (0.62-7.10)	0.231
BCLC stage	0, A vs. B, C, D	4.42 (2.91-6.70)	< 0.001*	3.00 (1.53-5.87)	0.001*
Plasma BAFF level (pg/ml)	< 1100 vs. ≥ 1100	3.10 (1.75-5.49)	< 0.001*	2.28 (1.07-4.87)	0.034*
rs9514828	CC vs. CT + TT	1.32 (0.52-3.32)	0.557		
rs12583006	AA vs. AT + TT	0.98 (0.57-1.67)	0.931		

Data express as odds ratio (OR) and 95% confidence intervals (CI); *, *P*-value < 0.05

distribution among studied groups.

The associations between these two SNPs and plasma BAFF levels were also examined. However, our data did not detect any significant difference of plasma BAFF levels in relation to different genotypes of rs9514828 or rs12583006 in all subjects. For example, average BAFF level of individuals with rs9514 828 CT and CT + TT was 1054.5 ± 630.2 and 1028.6 ± 469.6 pg/ml, respectively ($P = 0.580$), while average BAFF level of individuals with rs12583006 AA and AT + TT was 1113.2 ± 802.7 and 1023.2 ± 467.1 pg/ml, respectively ($P = 0.259$). Also, there was no such difference in subgroups of patients with HCC, patients without HCC and healthy controls (data not shown).

Factors associated with overall survival of patients with HCC

We further examined the potential prognostic value of plasma BAFF and its related SNPs. The median overall survival (OS) of patients with low levels of BAFF (< 1100 pg/ml) was 47.5 months, which was significantly better than that of patients whose levels were ≥ 1100 pg/ml (21.4 months, $P < 0.001$ by log rank test) (**Figure 2A**). For rs9514828, there was no difference in OS between patients harboring CC or CT + TT (**Figure 2B**). Similarly, there was no difference in OS between patients harboring AA or AT + TT of rs12583006 (**Figure 2C**).

Plasma BAFF, rs9514828 and rs12583006 were entered into multivariate analysis together with other variables that might influence OS of patients with HCC. These factors included age, gender, AST, ALT, platelet count, HBV DNA, FIB-4 index, Child-Pugh classification, tumor size and BCLC stage. The multivariate analysis revealed that more advanced BCLC (stage B,C,D vs stage 0,A) [odds ratio (OR) = 3.00, 95% confidence intervals (CI):1.53-5.87; $P = 0.001$], high AFP (≥ 100 vs. < 100 ng/ml) (OR = 3.64, 95%CI: 1.53-8.64; $P = 0.003$) and high plasma BAFF levels (≥ 1100 vs. < 1100 pg/ml) (OR = 2.28, 95%CI: 1.07-4.87; $P = 0.034$) were independent poor prognostic factors of OS in patients with HCC (**Table 4**).

Discussion

HCC represents a leading cancers worldwide, especially in Southeast Asia, where HBV is highly prevalent.[1] Overall, the prognosis of HCC is poor due to aggressive tumor characteristics and an advanced stage at presentation. The pathogenesis of HBV-related HCC is thought to be a multi-step process, which is mainly associated with immune-mediated liver injury.[2] Accumulating evidence has demonstrated the critical roles of adaptive immunity response to HBV infection that contributes in chronic liver inflammation leading to hepatocarcinogenesis, though most reports have focused on the effects of HBV-specific T cells.[22] Conversely, relatively little is currently known regarding the clinical importance of B-cell immunity in HCC progression and prognosis.

In this study, we confirmed previous data that circulating BAFF levels were significantly higher in patients with HCC compared with healthy individuals and patients with non-HCC.[14] Among patients without HCC, plasma BAFF levels had a gradually increase from inactive carriers (IC) to the immune active (IA) group and cirrhosis. Our data showed that plasma BAFF levels were positively correlated with serum ALT, a surrogate marker of liver injury. These data indicate that BAFF is increased during active inflammation, possibly as a consequence of BAFF production by its inducers such as type I

interferons.[5] Notably, among patients with immune active disease, BAFF levels were significantly higher in patients with HBeAg-positive that those with HBeAg-negative. Consistent with our report, previous data from a cell culture model demonstrated that HBeAg itself was capable of promoting BAFF activation through regulating monocyte function.[23] Our results also demonstrated that BAFF levels correlated with liver fibrosis assessed by FIB-4 index and their levels were significantly increased in patients with cirrhosis compared with the non-cirrhotic group, supporting previous findings that BAFF levels progressively increase in cirrhosis independent of underlying etiologies of liver disease.[22,24]

Our data showed that elevated plasma BAFF levels were significantly correlated with more aggressive tumor characteristics in patients with HBV-related HCC. In addition, a high BAFF level at initial presentation was associated with an unfavorable outcome in these patients. Specifically, a high plasma BAFF level was observed more frequently in patients with large tumor burden and advanced BCLC stages. Furthermore, multivariate analysis revealed that this marker was an independent, unfavorable predictor of survival in patients with HCC. Specifically, HCC patients with high circulating BAFF (≥ 1100 pg/ml) at initial presentation had approximately 2-fold increased risk of adverse outcome compared to patients with lower BAFF levels. These data strongly suggest that the prognosis of HCC is influenced by the extent of circulating BAFF expression. Collectively, these findings demonstrate that plasma BAFF may represent a useful biomarker in monitoring tumor progression and prognosis in patients with HBV-related HCC.

Similar findings of BAFF levels in association with tumor progression and prognosis were observed in hematological and non-hematological malignancies.[10,25-28] For instance, the levels of BAFF were positively correlated with disease severity, poor therapeutic response and adverse clinical outcome in patients with lymphoma.[10,25,26] In addition, BAFF levels were associated with disease activity and advanced disease stage in patients with multiple myeloma.[27] Moreover, circulating BAFF levels were highly expressed in patients with pancreatic cancer, especially among those with metastatic disease.[27] Significantly elevated circulating BAFF were also found in adolescent patients with certain types of sarcoma in relation to cancer-related cachexia.[28] These data highlight an essential and active role of BAFF in disease severity of HCC and other tumor types.

As BAFF has emerged a critical factor of peripheral B cell survival, it is likely that B cells may be contributable to disease progression and HCC development in patients with chronic HBV infection. To support this notion, a previous study directly demonstrated that B celldeficient mice displayed attenuated liver fibrosis induced by CCl4, representing a pro-fibrogenic activity of B cells.[29] A more recent report showed that intrahepatic B cells were responsible for hepatic stellate cell-mediated liver fibrosis through the production of several inflammatory cytokines.[30] Moreover, a recent study showed that B cells played a critical role in hepatocarcinogenesis following chronic liver injury.[31] In an animal model, elimination of B cells, but not T cells, could promote the resolution of liver fibrosis and prevent important signaling pathways towards HCC development. The role of B cells was also confirmed in patients with HCC demonstrating that increased infiltrating B cells within cancerous tissues was linked to poor tumor differentiation, advanced stages and reduced disease-free survival of HCC.[31] Likewise, increase percentage of B cells in PBMCs was also demonstrated in patients with more advanced tumor stages compared to those with early HCC.[26] Taken together, these data highlight the significance of B cells in modulating liver fibrogenesis and, more importantly, the development and progression of HCC.

Regarding the BAFF polymorphisms, it was previously shown that these genetic variations might result in changes of BAFF activity and expression, and was reported to be associated with the pathogenesis of autoimmune diseases, hematological malignancies or chronic infection.[15-17] For instance, the T allele of rs9514828 polymorphism in the BAFF promoter was more predominant in patients with HCV-related mixed cryoglobulinemia (MC) and associated with an increase in BAFF levels when compared with chronic HCV carriers without MC.[32,33] In this study, our data showed that the frequency of rs9514828 CT + TT genotypes was significantly higher distributed in patients with chronic HBV infection, including the HCC and non-HCC groups, compared with healthy controls. However, significant difference in their distributions between patients with HCC and non-HCC was not observed. Moreover, rs9514828 genotypes exhibited no association with plasma BAFF levels or other clinical parameters of patients with chronic HBV infection. These results suggested that CT + TT genotypes might be associated with susceptibility to HBV infection but not related to disease progression or HCC development in Thai populations. Of noted, our findings were partly in line with recent data demonstrating that these genotypes might confer susceptibility to chronic HBV infection in Chinese Han populations, probably not directly through circulating BAFF expression.[18] Regarding rs12583006, another polymorphism located in the noncoding region of BAFF, our results showed that this genetic variation did not have any influence on plasma BAFF levels and, more importantly, displayed no role on clinical significance in patients with chronic HBV infection.

This report might have some limitations. First, the study was a retrospective design and the sample size of patients with or without HCC was relatively small. Second, the analysis of genetic variations included only two polymorphisms and limited in Thai patients, which might not be applicable to other ethnic populations.

Conclusion

In conclusion, this is the first report demonstrates the clinical implications of plasma BAFF in patients with HBV-related HCC. We found that a high level of BAFF was significantly associated with tumor progression and invasiveness. Moreover, elevated plasma BAFF level was an independent prognostic factor of overall survival. These findings have clinical implications as plasma BAFF at initial diagnosis could serve as a prognostic marker for patients with HBV-related HCC. Also, these data might indicate that B cell immunity is contributable to the development and progression of HCC in patients with chronic HBV infection. Further studies are, however, required to validate these observations in patients with HCC regardless of underlying etiologies and to elucidate the mechanistic roles of B cell-mediated immune response in hepatocarcinogenesis.

Acknowledgements

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Conflict of Interest

All authors declare no conflict of interest.

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ORIGINAL ARTICLE

Circulating BAFF and CXCL10 levels predict response to pegylated interferon in patients with HBeAg-positive chronic hepatitis B

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Abstract

Background: B-cell activating factor (BAFF), an essential cytokine for B lymphocytes activation, has been implicated in the pathogenesis of chronic viral hepatitis. However, the role of BAFF in patients with chronic hepatitis B (CHB) undergoing antiviral therapy is unknown.

Methods: Patients with HBeAg-positive CHB treated with 48-week pegylated interferon (PEG-IFN; n = 42), who had stored plasma samples during treatment were recruited. Serial plasma levels of BAFF and C-X-C motif chemokine 10 (CXCL10) during therapy were measured.

Results: Combined response (CR), defined as HBeAg seroconversion with HBV DNA < 2,000 IU/mL plus HBsAg decline ≥ 1 $\log_{10}$ IU/mL at 24 weeks post-treatment, was achieved in 11 (26.2%) patients. BAFF levels were elevated during treatment but decreased to pre-treatment levels after PEG-IFN cessation in both responders and non-responders. Low baseline BAFF (< 770 pg/ml) and high CXCL10 (≥ 320 pg/ml) levels were independently associated with CR in multivariate analysis. Baseline CXCL10/BAFF ratio of ≥ 0.45 was predictive of CR with positive and negative predictive values of 61.5 and 89.7%, respectively.

Conclusions: In summary, low baseline BAFF and high CXCL10 levels were associated with treatment response to PEG-IFN. The combined measurement of these immune markers may help individualized decision-making in patients with HBeAg-positive CHB.

Key words: BAFF, B cells, APRIL, CXCL10, IP-10, Peginterferon, hepatitis B, HBsAg

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Introduction

Chronic hepatitis B virus (HBV) infection is associated with diverse clinical manifestations including chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC).[1] Current therapeutic options for chronic hepatitis B (CHB) include oral nucleoside/nucleotide analogues (NA) and pegylated (PEG) in terferon (IFN). Compared to NA, PEG-IFN offers a finite duration of therapy and is associated with higher rates of sustained off-treatment response and hepatitis B surface antigen (HBsAg) clearance.[2] However, the use of PEG-IFN is hampered by potential adverse events. Therefore, a biomarker that could predict a high likelihood of PEG-IFN responsiveness would be highly desirable.

Current evidence has indicated that the outcome of HBV infection is determined by immune-mediated host-virus interactions.[3] Indeed, effective control of HBV infection involves the coordinated actions of both innate and adaptive immune responses. At early onset of acute infection, vigorous and specific B and T-cell responses participate in the process of viral clearance and self-limited liver injury. Conversely, the immune activity is functionally-impaired in individuals evolving to chronic HBV infection. It is well recognized that HBV-specific T cells play a major role in the efficacy of the adaptive immune response and ultimately determine clinical outcome of HBV infection.[4] In contrast to accumulating data of T-cell immunity, less is known about the role of B-cell-mediated immune response in the pathogenesis and treatment outcome of CHB.

B cells play an important role in humoral immunity by producing antibodies, inducing immunomodulatory cytokines and influencing the T-cell response.[5] Differentiation and proliferation of B cells are regulated by various cytokines such as a proliferation-inducing ligand (APRIL) and B cell-activating factor (BAFF, also known as B Lymphocyte Stimulator (BLys) or TNF- and APOL-related leukocyte expressed ligand (TALL-1). BAFF, a member of the tumor necrosis factor superfamily (TNFSF13B) and an IFN stimulated gene, is produced by many cell types including monocytes, macrophages, dendritic cells, neutrophils and activated T cells.[6] The expression of BAFF is stimulated by interferon-gamma (IFN-γ), interleukin (IL)-10 and CD40 ligand.[6] Previous studies have shown that circulating BAFF is increased in several autoimmune and chronic inflammatory disorders.[7-9] Elevated BAFF levels is also found in patients with hepatitis C virus (HCV) infection, particularly in individuals with cryoglobulinemia.[10,11] IFN-based treatments up-regulated BAFF levels in patients with chronic HCV infection, especially in those achieving viral clearance.[11] Additionally, a recent study has showed that BAFF concentration was elevated in patients with CHB in comparison with healthy controls and the level of BAFF was associated with unfavorable clinical consequences including cirrhosis and HCC.[12] Together, these results suggest that BAFF may contribute to the pathogenesis of chronic viral hepatitis and may potentially predict treatment outcome in patients with CHB.

The aim of this study was to determine the relationship between plasma BAFF levels and treatment response following PEG-IFN in patients with HBeAg-positive CHB. In conjunction with BAFF levels, we also examined plasma concentrations of APRIL and CXCL10, a marker of IFN-stimulated genes (ISG).[13] Our data clearly demonstrated that measuring both BAFF and CXCL10 at baseline might facilitate individualized decision-making before initiating PEG-IFN therapy.

Methods

Patients

Forty-two patients with HBeAg-positive CHB, who were treated with 48-week PEG-IFN-alfa2a (180 µg/week) between January 2010 and May 2015 and followed up for at least 24 weeks after therapy at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand were enrolled. These patients had available stored plasma samples at baseline and during treatment. All these patients had HBsAg positivity, elevated serum alanine aminotransferase (ALT) and serum HBV DNA levels for at least 6 months before therapy. Patients with HCV and/or human immunodeficiency virus (HIV) co-infection were excluded. Virological response (VR) was defined as HBeAg seroconversion (HBeAg clearance and generation of anti-HBe) plus HBV DNA level < 2,000 IU/mL at 24 weeks after complete treatment. Combined response (CR) was defined by VR plus HBsAg decline ≥ 1.0 $\log_{10}$ IU/mL at 24 weeks post treatment.

The study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and participants had provided written informed consent. The study followed the Helsinki Declaration and Good Clinical Practice guidelines.

Serological and virological assays

Qualitative measurements of HBsAg, HBeAg and Anti-HBe were tested by commercial available enzyme-linked immunosorbent assays (Abbott Laboratories, Chicago, IL). Serum HBsAg quantification was assessed by Elecsys HBsAgII Quant reagent kits (Roche Diagnostics, Indianapolis, IN) and HBV DNA levels were tested by Abbott Real Time HBV assay (Abbott Laboratories). HBV genotyping and mutations in the precore (PC, G1896A) and basal core promoter (BCP; A1762T and/or G1764A)) regions were assessed by direct sequencing, as described previously.[14] Patients were then classified as being infected with wild type (WT) or mutant HBV.

Enzyme-linked immunosorbent assays

Plasma BAFF and CXCL10 levels were determined by ELISA (R&D Systems, Minneapolis, MN) at baseline, during and after therapy (weeks 0, 4, 12, 24, 48 and 72). Plasma APRIL was measured at baseline using Human APRIL Platinum ELISA (eBioscience, Vienna, Austria) according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed with SPSS statistics version 22 (SPSS Inc., Chicago, IL) and GraphPad Prism v5.0 (GraphPad Software, San Diego, CA). Values are presented as mean ± standard deviation (SD), and percentages as appropriate. Comparisons between groups were assessed by the $\chi 2$ or Fisher's exact test for categorical variables and by the Mann–Whitney *U*-test or Student's *t*-test for quantitative variables. Spearman correlation coefficient was applied to evaluate the correlation between baseline parameters. Areas under the receiver operating characteristic curve (ROC) were used to assess the predictive values of variables for treatment response. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated in accordance with standard methods. Univariate and multivariate logistic regression were used to assess odd ratios relating pre-treatment variables associated with treatment response. A p value < 0.05 was considered statistically significant.

Results

Baseline patient characteristics

In this cohort, 17 (40.5%), 11 (26.2%) and 3 (7.1%) patients achieved VR, CR and HBsAg clearance, respectively. Baseline

characteristics of all patients and those with and without CR are shown in **Table 1**. Patients who achieved CR (responders) had significantly lower baseline BAFF concentrations but had significantly higher baseline CXCL10 levels than non-responders. In addition, responders had lower frequencies of PC and BCP mutations than non-responders. There was no significant difference between groups in the distribution of patient's gender, HBV genotypes, mean HBV DNA, HBsAg and APRIL levels. Baseline characteristics of patients in relation to VR and HBsAg clearance are shown in **Supplement table 1**.

Baseline levels of BAFF, APRIL and CXCL10 in relation to treatment outcome are shown in **Figure 1**. For BAFF concentrations, patients with VR compared to those without VR had a significant lower mean baseline level (762.4 ± 199.7 vs. 923.7 ± 231.1 pg/mL, P = 0.024). Similar findings were observed in relation to patients with and without CR (722.6 ± 208.6 vs. 906.6 ± 221.6 pg/mL, P = 0.021) and with and without HBsAg clearance (556.3 ± 77.7 vs. 881.6 ± 222.2 pg/mL, P = 0.017). For APRIL levels, the corresponding figures were as following: VR vs no VR (2.3 ± 2.4 vs. 6.5 ± 11.7 pg/mL, P = 0.131), CR vs no CR (2.5 ± 2.8 vs. 5.7 ± 10.8 pg/mL, P = 0.368) and HBsAg clearance vs no clearance (1.0 ± 0.2 vs. 5.1 ± 9.6 pg/mL, P = 0.472). Regarding baseline CXCL10, levels at baseline differed between VR vs non VR (493.9 ± 328.0 vs. 262.3 ± 114.1 pg/mL, P = 0.012) and CR vs non CR (562.5 ± 371.6 vs. 282.8 ± 136.7 pg/mL, P = 0.033). However, there were no significant

Table 1. Baseline characteristics of patients in relation to combined response

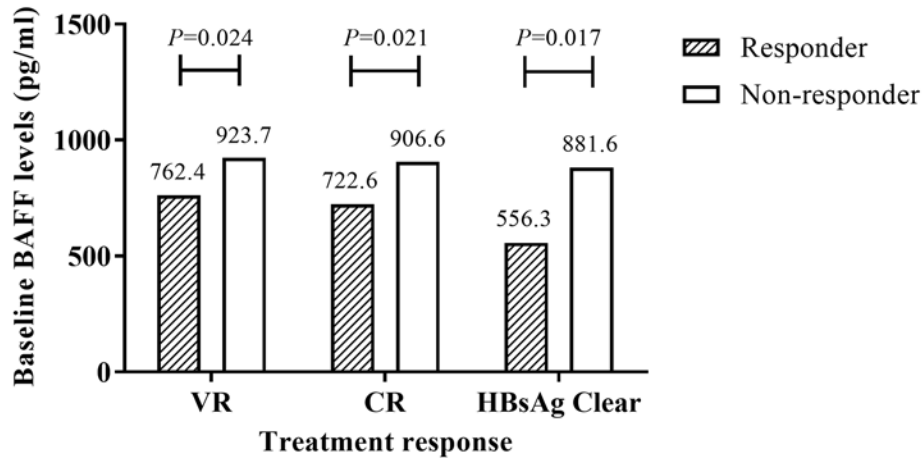
Characteristics	All patients (n = 42)	Responders (n = 11)	Non-responders (n = 31)	*P* value
Age, year	33.8 ± 8.2	32.7 ± 8.4	34.2 ± 8.2	0.609
Male sex, n (%)	28 (66.7%)	7 (63.6%)	21 (67.7%)	0.804
ALT, U/l	97.2 ± 71.5	93.9 ± 59.2	98.3 ± 76.3	0.863
HBV genotypes, n (%)				
B	5 (11.9%)	1 (9.1%)	4 (12.9%)	0.737
C	37 (88.1%)	10 (90.9%)	27 (87.1%)	
PC and BCP Mutation, n (%)	21 (50%)	2 (18.2%)	19 (61.3%)	0.014*
Log_{10} HBV DNA, IU/ml	7.2 ± 1.1	7.4 ± 1.2	7.1 ± 1.1	0.541
Log_{10} HBsAg, IU/ml	3.9 ± 0.7	4.1 ± 0.8	3.9 ± 0.7	0.371
BAFF, pg/ml	858.4 ± 230.7	722.6 ± 208.6	906.6 ± 221.6	0.021*
APRIL, ng/ml	4.8 ± 9.3	2.5 ± 2.8	5.7 ± 10.8	0.368
CXCL10, pg/ml	356.0 ± 250.7	562.5 ± 371.6	282.8 ± 136.7	0.033*

Values are presented as means ± SD unless otherwise specified.
ALT, alanine aminotransferase; PC, Precore; BCP, Basic core promoter; Responders, patients achieved combined response

Supplement Table 1.

Characteristics	Virological response		*P* value	HBsAg clearance		*P* value
	Yes (n = 17)	No (n = 25)		Yes (n = 3)	No (n = 39)	
Age, year	33.0 ± 8.3	34.4 ± 8.3	0.594	26.7 ± 5.5	34.4 ± 8.2	0.118
Male sex, n (%)	12 (70.6%)	16 (76.2%)	0.705	2 (66.7%)	26 (66.7%)	1.000
ALT, U/l	92.7 ± 57.4	100.2 ± 80.8	0.727	126.0 ± 84.1	94.9 ± 71.3	0.591
HBV genotypes, n (%)						
B	3 (17.6%)	2 (8.0%)	0.379	0 (0%)	5 (12.8%)	0.509
C	14 (82.4%)	23 (92.0%)		3 (100%)	34 (87.2%)	
PC and BCP Mutation, n (%)	5 (29.4%)	16 (64.0%)	0.028*	0 (0%)	21 (53.8%)	0.072
Log_{10} HBV DNA, IU/ml	7.1 ± 1.2	7.3 ± 1.1	0.575	8.2 ± 0.3	7.1 ± 1.1	0.116
Log_{10} HBsAg, IU/ml	3.8 ± 0.8	4.0 ± 0.7	0.478	4.7 ± 0.0	3.8 ± 0.7	0.044*
BAFF, pg/ml	762.4 ± 199.7	923.7 ± 231.1	0.021*	556.3 ± 77.7	881.6 ± 222.2	0.017*
APRIL, ng/ml	2.3 ± 2.4	6.5 ± 11.7	0.131	1.0 ± 0.2	5.1 ± 9.6	0.472
CXCL10, pg/ml	493.9 ± 328.0	262.3 ± 114.1	0.012*	734.5 ± 552.5	326.9 ± 198.9	0.005*

Values are presented as means ± SD unless otherwise specified.
ALT, alanine aminotransferase; PC, Precore; BCP, Basic core promoter



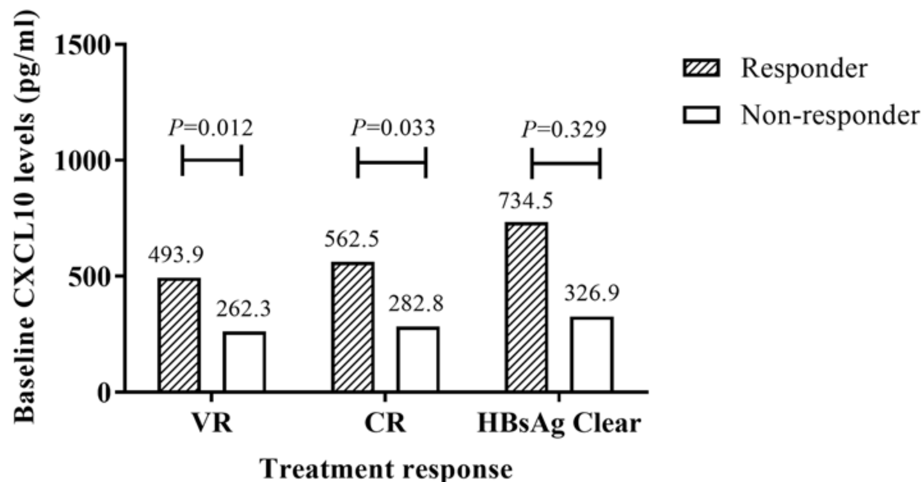


Figure 1. Baseline levels of (A) BAFF (B) CXCL10 in relation to treatment response

differences with and without HBsAg clearance (734.5 ± 552.5 vs. 326.9 ± 198.9 pg/mL, P = 0.329).

Baseline plasma BAFF levels were positively correlated with baseline plasma APRIL levels (r = 0.471, P = 0.005) but there was no correlation with plasma CXCL10 levels (r = 0.04, P = 0.801), HBV DNA, HBsAg or ALT levels. Baseline CXCL10 levels correlated with ALT levels (r = 0.369, P = 0.016), but did not correlated with HBV DNA and HBsAg concentrations.

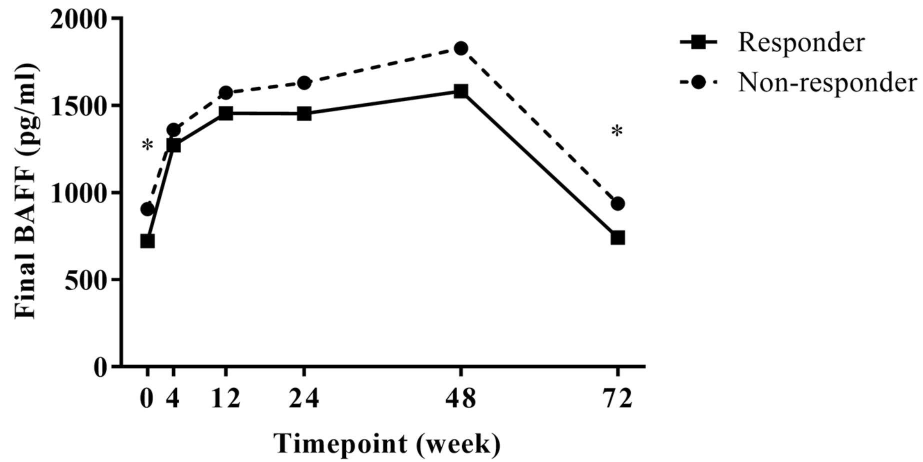
Plasma BAFF and CXCL10 kinetics in relation to combined response

Regardless of treatment response, plasma BAFF levels were significantly elevated after the beginning of PEG-IFN therapy and decreased after the end of treatment (**Figure 2A**). Mean BAFF levels were significantly different between those with and without CR at week 0 (722.6 ± 208.6 vs. 906.6 ± 221.6 pg/mL, P = 0.021) and week 72 (742.3 ± 219.8 vs. 938.3 ± 228.4 pg/mL, P = 0.018). However, there were no significant differences between groups at other time points. In addition, the mean changes from baseline at weeks 4, 12, 24, 48 and 72 were not different between responders and non-responders.

For circulating CXCL10, individuals with a CR compared to no CR had higher levels at baseline (562.5 ± 371.6 vs. 282.8 ± 136.7 pg/mL, P = 0.033) and week 12 (603.4 ± 175.1 vs. 431.6 ± 141.3 pg/mL, P = 0.002) but not at week 72 (162.5 ± 70.7 vs. 235.9 ± 152.1 pg/mL, P = 0.133) (**Figure 2B**). The results showed no difference between groups at other time points. Considering the dynamic changes from baseline, the mean decline of CXCL10 levels was significantly different between individuals with a CR compared to no CR at week 24 (202.3 ± 409.2 vs. -89.9 ± 109.2 pg/mL, P = 0.042), week 48 (240.7 ± 377.0 vs. -20.2 ± 156.8 pg/mL, P = 0.047) and week 72 (400.0 ± 399.0 vs. 46.8 ± 163.8 pg/mL, P = 0.015).

Cut-off values of baseline BAFF and CXCL10 in predicting combined response

The cut-off values of BAFF and CXCL10 for predicting CR are shown in **Supplement Figure 1**. The area under ROC curves (AUROC) of BAFF and CXCL10 were 0.74 (95% confidence interval (CI), 0.55-0.93; P = 0.018) and 0.77 (95%CI, 0.60-0.94; P = 0.008), respectively. The optimal cut-off values for BAFF and CXCL10 were 770 and 320 pg/mL, respectively. For baseline



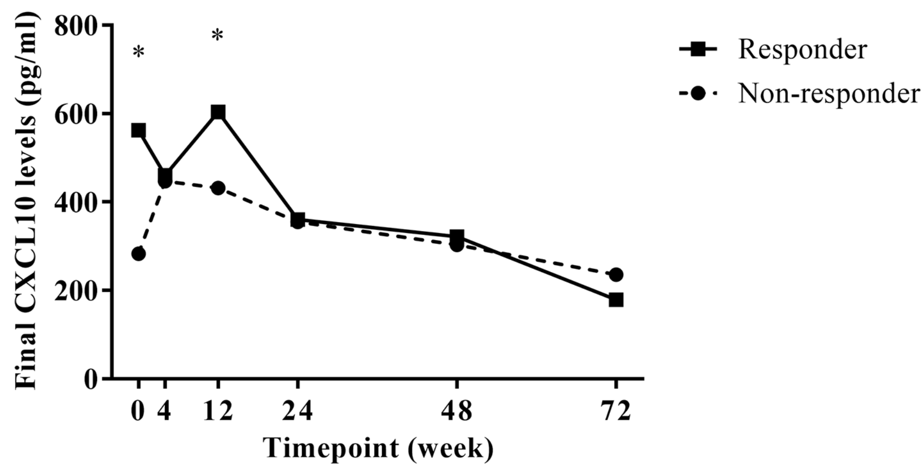
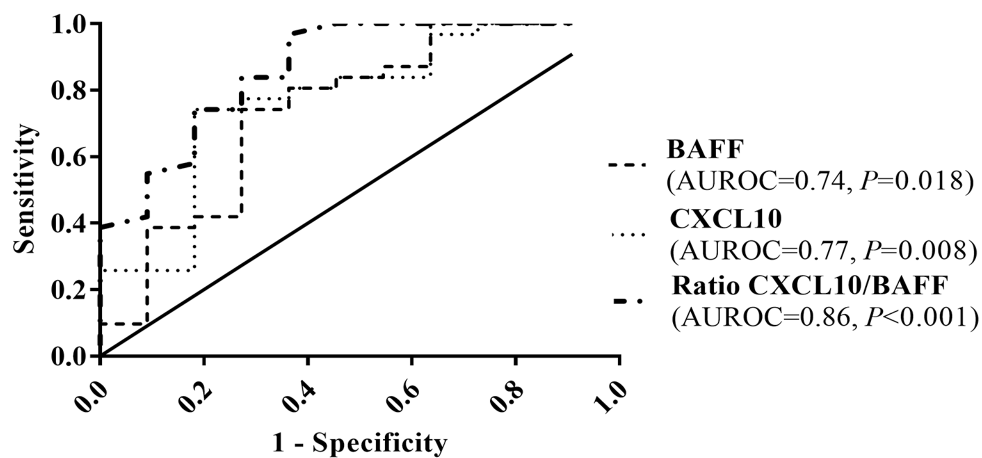


Figure 2. Kinetics of BAFF and CXCL10 levels during PEG-IFN therapy. (A) BAFF (B) CXCL10 *P < 0.05



Supplement Figure 1.

circulating APRIL, the AUROC was 0.62 (95%CI, 0.40-0.84; P = 0.273) and the best cut-off level was 1.30 ng/mL. The sensitivity, specificity, PPV, NPV and accuracy for the prediction of CR of these markers are shown in **Table 2**.

As the expression of circulating BAFF and CXCL10 exhibited an opposite pattern, we further analyzed the ratio of CXCL10 to BAFF levels. The AUROC of CXCL10/BAFF ratio was 0.86 (95%CI, 0.73-0.99; P < 0.001. Based on ROC analysis, the best cut-off point of CXCL10/BAFF ratio was 0.45. At this optimal value, the sensitivity, specificity, PPV, NPV and accuracy for predicting CR were 72.7, 83.9, 61.5, 89.7 and 81.0, respectively (**Table 2**).

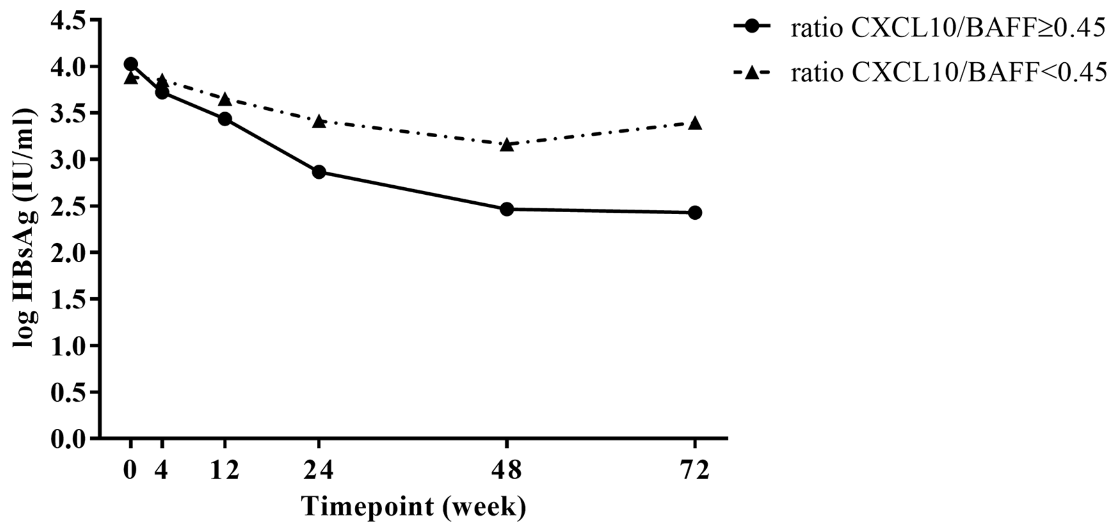
HBsAg kinetics in relation to baseline CXCL10/BAFF ratio

To compare HBsAg kinetics in relation to baseline CXCL10/BAFF ratio, the best cut-off value of 0.45 was applied. Patients with a high ratio (≥ 0.45; n = 14) compared with those with low ratio (< 0.45; n = 28) had similar levels of HBsAg (4.0 ± 0.7 vs. 3.9 ± 0.7 log_{10}IU/mL, P = 0.561) but a trend towards a greater HBsAg decline from baseline: week 4 (0.3 ± 0.5 vs. 0.1 ± 0.3 log_{10}IU/mL, P = 0.060), week 12 (0.6 ± 0.7 vs. 0.2 ± 0.3 log_{10}IU/mL, P = 0.097), week 24 (1.2 ± 1.3 vs. 0.5 ± 0.6 log_{10}IU/mL, P = 0.088), week 48 (1.6 ± 1.6 vs. 0.7 ± 0.9 log_{10}IU/mL, P = 0.086) and week 72 (1.6 ± 1.8 vs. 0.5 ± 1.1 log_{10}IU/mL, P = 0.045) (**Supplement Figure 2**).

Table 2. Cut-off levels of parameters to predict combined response

Parameters	Cut-off values	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
BAFF	770 pg/ml	72.7	71.0	47.1	88.0	71.4
CXCL10	320 pg/ml	72.7	74.2	50.0	88.5	73.8
CXCL10/BAFF ratio	0.45	72.7	83.9	61.5	89.7	81.0

NPV, negative predictive value; PPV, positive predictive value



Supplement Figure 2.

Table 3. Logistic regression analysis of baseline characteristics to predict combined response

Factors	Categories	Combined response			
		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age, year	< 40 vs. ≥ 40	1.1 (0.2-5.1)	0.912		
Sex	Male vs. Female	0.8 (0.2-3.5)	0.804		
ALT, IU/ml	< 100 vs. ≥ 100	0.9 (0.2-4.3)	0.912		
HBV genotypes	B vs. C	0.7 (0.1-6.8)	0.739		
PC/BCP mutants	Wild type vs. Mutants	7.1 (1.3-38.8)	0.023*	4.2 (0.5-33.8)	0.175
Log_{10} HBV DNA, IU/ml	< 7.0 vs. ≥ 7.0	4.2 (0.8-22.8)	0.094		
Log_{10} HBsAg, IU/ml	< 4.0 vs. ≥ 4.0	2.4 (0.6-10.0)	0.222		

ALT, alanine aminotransferase; PC, Precore; BCP, Basic core promoter; OR, odd ratio; CI, confident interval

Table 3. (Continued)

Factors	Categories	Combined response			
		Univariate analysis		Multivariate analysis	
		OR (95% CI)	*P* value	OR (95% CI)	*P* value
APRIL, ng/ml	< 1.3 vs. ≥ 1.3	2.5 (0.6-11.3)	0.235		
BAFF, pg/ml	< 770 vs. ≥ 770	7.7 (1.6-36.2)	0.010*	16.1 (1.5-174.9)	0.022*
CXCL10, pg/ml	≥ 320 vs. < 320	12.9 (2.3-73.0)	0.004*	24.2 (2.1-280.6)	0.011*

ALT, alanine aminotransferase; PC, Precore; BCP, Basic core promoter; OR, odd ratio; CI, confident interval

Predictors of combined response at baseline

Univariate and multivariate analyses were performed in order to identify pre-treatment predictors of CR. Selected baseline factors included sex, age, ALT, HBV genotype, viral mutations, HBV DNA, HBsAg, plasma BAFF, APRIL and CXCL10 levels. In univariate analysis, parameters associated with CR were the presence of WT virus (no PC and/or BCP mutants), low plasma BAFF (< 770 pg/ml) and high CXCL10 (≥ 320 pg/ml) levels. In multivariate analysis, only low BAFF and high CXCL10 levels were independent predictors for CR (**Table 3**).

Discussion

The ultimate but difficult to achieve end-point in the management of patients with HBeAg-positive CHB is HBsAg clearance/seroconversion, which is considered to be a functional cure resulting in favorable long-term clinical outcomes including reduced rates of cirrhosis and HCC development.[15] HBsAg clearance was observed in approximately 7% of our report, a rate comparable to previous data in patients with HBeAg-positive CHB treated with PEG-IFN (3-7%).[1] Given the low rate of achieving HBsAg clearance after PEG-IFN therapy, a well-recognized and more realistic goal in clinical practice is HBeAg seroconversion with sustained virological suppression (VR). In the natural history of CHB, however, HBV DNA levels fluctuate more often than HBsAg levels. Thus, low HBV DNA level at a single time point might not guarantee persistently viral suppression. Another valuable parameter reflecting effective immunity following antiviral therapy is a reduction in serum HBsAg concentrations. Indeed, significant HBsAg decline represents an immune control of chronic HBV infection, helps differentiate patients likely to achieve sustained off-treatment response and offers a good prediction of subsequent HBsAg clearance in long-term follow-up.[16] Based on this concept, we used a combined response of VR plus a significant decline of HBsAg level as the main therapeutic outcome in this cohort.

In the present study, we aimed at investigating whether baseline and on therapy kinetics of plasma CXCL10 and BAFF levels were associated with treatment response to PEG-IFN in Thai patients with HBeAg-positive CHB. Our data clearly demonstrated that high circulating CXCL10 level prior to treatment was positively correlated with increased likelihood of achieving CR. In contrast, an increased baseline BAFF level was negatively correlated with treatment outcome. Considering the reciprocal relationship between CXCL10 and BAFF levels, the calculation of baseline CXCL10/BAFF ratio could increase the sensitivity for predicting a treatment response. Based on the best cut-off value, the ratio of 0.45 displayed a PPV and NPV of approximately 62% and 90%, respectively. These results indicate that CXCL10/BAFF ratio may be applicable to individualize decision-making before initiation of PEG-IFN therapy in patients with HBeAg-positive CHB.

The novel finding in this study was the strong association of low plasma BAFF levels at the start of PEG-IFN with a successful treatment response. Lower baseline BAFF levels were associated with a rapid decline in HBsAg levels and higher rates of HBsAg clearance at the end of follow-up. Interestingly, similar findings in patients with chronic HCV infection also demonstrated that responders to IFN-based therapy had lower pre-treatment BAFF levels than non-responders.[10] Moreover, BAFF levels were significantly higher in patients with acute HCV infection evolving to chronicity than in those with a self-limited course.[17] In patients with CHB, a stepwise elevated BAFF concentrations were correlated with disease severity included cirrhosis and HCC.[12]

Of note, our data showed that baseline plasma BAFF concentrations did not correlate with circulating CXCL10 levels. However, plasma BAFF levels gradually increased during PEG-IFN therapy and decreased to levels similar to baseline after cessation of the treatment. These findings indicated that the up-regulation of BAFF was mainly regulated by the effect of PEG-IFN, similar to previous reports in patients with chronic HCV infection undergoing IFN-based therapy.[10,11,17] In contrast to pre-treatment levels, the kinetics of BAFF was not correlated with treatment outcome as responders and non-responders had comparable pattern and dynamic changes in BAFF concentrations during PEG-IFN therapy. As a consequence, monitoring on-treatment BAFF might not provide additional information in predicting PEG-IFN responsiveness in our cohort.

The mechanisms by which baseline BAFF concentrations modulate therapeutic outcome of PEG-IFN in patients with HBeAg-positive CHB are largely unknown. BAFF has emerged as a cytokine that plays an essential role in B cell proliferation, differentiation, survival and antibody production.[6] By secreting neutralizing antibodies, B cells are able to minimize viral spread and contribute to viral elimination. In addition, B cells are capable of acting as antigen-presenting cells and modulating T cell responses. During HBV infection, the process of B-cell activation and its interaction with HBV-specific T cells is considered to be crucial for diverse clinical outcomes of infected individuals.[4] It has been shown that vigorous T cell responses induce B cell activation, which in turn leads to anti-viral T cell responsiveness and favors neutralizing antibody formation.

In contrast, the interaction of B cells and T cells could also up -regulate the expression of PD-1, a hallmark of T cell exhaustion during chronic viral infection.[18] Recent data have shown that total B-cell hyper-activation but impaired generation of HBV-specific B-cells are commonly found in chronic HBV infection and reversal of these processes is associated with HBsAg seroconversion.[19] Moreover, it has been demonstrated in a cell culture model that HBeAg itself is able to regulate monocyte function and promote BAFF activation.[20] Given these observations, we propose that high BAFF concentrations found in this study might reflect B-cell hyper-activation, thereby altering T cell function and reducing response to PEG-IFN in patients with HBeAg-positive CHB. A better understanding of the mechanism by which BAFF and B cells modulate T-cell function in the presence and absence of PEG-IFN in patients with CHB requires further investigations.

CXCL10 is a pro-inflammatory chemokine that plays an essential role in the pathogenesis of chronic viral hepatitis. Upon its binding to the chemokine receptor 3 (CXCR3), CXCL10 activates T lymphocytes and natural killer (NK) cells undergo chemotaxis.[13] In HBV infection, increased intrahepatic expression of CXCL10 leads to accumulation of inflammatory cells, which results in the activation of immune-mediated liver injury.[21,22] It has been shown in an animal model of transgenic mice that inhibition of CXCL10 significantly reduces the recruitment of inflammatory cells and severity of liver damage.[23] Elevated circulating CXCL10, which is correlated with high intrahepatic CXCL10 expression, has also been shown to be correlated with the degree of liver inflammation and fibrosis in HBV-infected individuals.[24] In patients with CHB, high baseline circulating CXCL10 levels have a predictive value of response to PEG-IFN or NA therapy.[25-30] In addition, CXCL10 is an ISG and therefore would be expected to increase following PEG-IFN. In line with these reports, our data confirmed that higher CXCL10 levels were associated with favorable outcome of PEG-IFN therapy. Given positive correlation of baseline CXCL10 levels with therapeutic outcome, increased circulating CXCL10 concentrations might represent a pre-existing active immunity attributable to higher response rates in patients with CHB.

Interestingly, we also found that dynamic decline of CXCL10 was associated with an increased likelihood of treatment response during PEG-IFN therapy. Specifically, decreasing CXCL10 levels from baseline was significantly higher in patients achieving CR. However, such finding was not detected among non-responders. Indeed, a similar trend was also observed in previous studies demonstrating that down-relation of circulating CXCL10 is more pronounced among responders than non-responders treated with PEG-IFN or NA.[26,30] As CXCL10 expression in HBV infection primarily contributes to liver injury, [31] significant decline in responders during and after treatment could reflect an improved immune control of HBV infection after successful antiviral therapy.

Although this is the first demonstration of the role of BAFF in predicting a treatment response in CHB, there were several limitations. First, the sample size was relatively small. Second, the study was retrospective but this might not lead to any confounding of the results because BAFF levels were significantly increase during therapy and then decreased to baseline after PEG-IFN cessation. Moreover, the present study included only patients with HBeAg- positive CHB but did not recruit patients with HBeAg-negative CHB.

Conclusion

In summary, our results strongly showed that baseline CXCL10 and BAFF levels were predictive of a clinically relevant response to PEG-IFN in Thai patients with HBeAg -positive CHB. Thus, combined measurement of these biomarkers of immune activity prior to PEG-IFN not only would motivate patients to adhere to treatment but also could maximize therapeutic cost-effectiveness. As the sample size of patients enrolled in this study was limited, a replicate study with larger number of patients is needed to verify these observations and would provide further insights into the role of BAFF and B cell response in patients with CHB.

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Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to the manuscript.

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