Cytokine 90 (2017) 54–59

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Circulating visfatin level is associated with hepatocellular carcinoma in chronic hepatitis B or C virus infection

I-Ting Tsai [a,g], Chao-Ping Wang [b,f], Teng-Hung Yu [b], Yung-Chuan Lu [c,f], Chih-Wen Lin [d], Li-Fen Lu [e], Cheng-Ching Wu [b,h], Fu-Mei Chung [b], Yau-Jiunn Lee [i], Wei-Chin Hung [b,*,1], Chia-Chang Hsu [d,*,1]

[a] Department of Emergency Medicine, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan
[b] Division of Cardiology, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan
[c] Division of Endocrinology and Metabolism, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan
[d] Division of Gastroenterology and Hepatology, Department of Internal Medicine, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan
[e] Division of Cardiac Surgery, Department of Surgery, E-Da Hospital, I-Shou University, Kaohsiung 82445, Taiwan
[f] School of Medicine for International Students, I-Shou University, Kaohsiung 82445, Taiwan
[g] Department of Nursing, I-Shou University, Kaohsiung 82445, Taiwan
[h] Institute of Biomedical Engineering, National Cheng Kung University, Tainan 70101, Taiwan
[i] Lee's Endocrinologic Clinic, Pingtung 90000, Taiwan

ARTICLE INFO

Article history:
Received 10 May 2016
Received in revised form 29 September 2016
Accepted 12 October 2016

Keywords:
Adipocytokines
Visfatin
Hepatocellular carcinoma
Chronic hepatitis B
Chronic hepatitis C

ABSTRACT

Adipocytokines play an important role in adipose tissue homeostasis, especially in obesity-associated disorders such as non-alcoholic fatty liver and their complications including hepatocellular carcinoma (HCC). Although visfatin is an adipocytokine highly expressed in visceral fat that has been demonstrated to play a critical role in the progression of human malignancies, little is known about the role of visfatin in HCC associated with chronic hepatitis C virus (HCV) and hepatitis B virus (HBV) infection. In this study, we investigated whether plasma visfatin levels were altered in patients with HCC and the association between plasma visfatin levels and pretreatment hematologic profiles. Plasma visfatin levels were measured by enzyme-linked immunosorbent assays in 193 patients with different stages of HBV or HCV infection, and 92 healthy control subjects. The patients with HCC and chronic HCV or HBV infection had higher levels of visfatin than patients with HBV, HCV, and cirrhosis. In multivariate logistic regression analysis, levels of alpha-fetoprotein (AFP) (OR: 1.13, p = 0.003), and plasma visfatin (OR: 1.17, p = 0.046) were independently associated with HCC. Multiple stepwise regression analysis showed that plasma visfatin level was positively associated with age, aspartate aminotransferase to platelet ratio index (APRI), and AFP. Trend analyses confirmed that plasma visfatin concentration was associated with AFP > 8 ng/mL, cirrhosis, HCC, tumor size > 5 cm, and Barcelona Clinic Liver Cancer-C stage. These results suggested that the plasma visfatin level is associated with the presence of HCC, and that a higher plasma visfatin level may be important in the pathogenesis of HCC. Visfatin may act as both a protective and pro-inflammatory factor. Plasma visfatin concentration may serve as an additional tool to identify patients with more advanced necroinflammation.

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infect hundreds of millions of people worldwide, and cause a spectrum of chronic liver diseases [1,2]. In up to 20% of patients, chronic HCV or HBV infection causes progressive hepatic fibrosis and cirrhosis, and approximately 10–20% of cirrhotic patients may develop hepatocellular carcinoma (HCC) within 5 years [3,4]. Previous studies have demonstrated that chronic HCV and HBV infections are important causes of HCC, being associated with more than 80% of cases worldwide [5], and epidemiological studies indicate that

* Corresponding authors at: Division of Gastroenterology and Hepatology, Department of Internal Medicine, E-Da Hospital, I-Shou University, No. 1, Yi-Da Rd., Jiau-Shu Village, Yan-Chao Township, Kaohsiung 82445, Taiwan (C.-C. Hsu). Division of Cardiology, Department of Internal Medicine, E-Da Hospital, I-Shou University, No. 1, Yi-Da Rd., Jiau-Shu Village, Yan-Chao Township, Kaohsiung 82445, Taiwan (W.-C. Hung).
E-mail addresses: hwc.cct@msa.hinet.net (W.-C. Hung), p751021@hotmail.com (C.-C. Hsu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cyto.2016.10.007
1043-4666/

the mortality rate is rising [6]. Hence, investigating the risk factors for the development of HCC in patients infected with HCV or HBV is of the utmost importance.

HCV- and HBV-related carcinogenesis has been reported to originate from chronic hepatitis, and to progress to HCC via a multistep process lasting for as long as 30 years [7]. During the progression of HCC, environmental factors (aflatoxin B1, alcohol consumption, cigarette smoking, hepatotoxic chemical agents) and many co-factors (elevated serum androgen levels, genetic polymorphisms, DNA repair enzymes) may act synergistically and lead to the progressive accumulation of multiple genomic changes in hepatocytes [8,9]. Obesity and associated metabolic abnormalities such as adipocytokine imbalance are known risk factors for the development of HCC. In addition, Kukla et al. reported that serum visfatin levels were significantly higher in patients with chronic hepatitis C (CHC), indicating that visfatin may play a critical role in the regulation of the inflammatory process in CHC [10]. Furthermore, Ninomiya et al. reported that visfatin plays an important role in inducing HCC cell proliferation, and that it may be directly related with the progression of this malignancy [11].

The potential mechanisms by which visfatin may cause carcinogenesis in patients with chronic HCV or HBV infection remain unclear. Several studies have reported that adipocytokine dysregulation can induce the pathological state of liver diseases [12–14], and that visfatin, which is an adipocytokine is expressed in normal, inflamed, and tumor tissues [15,16]. Visfatin possesses nicotinamide adenine dinucleotide (NAD) biosynthetic activity, and it has been shown to regulate growth, apoptosis, and angiogenesis in mammalian cells [17,18]. It was originally identified as a pre-B-cell colony-enhancing factor, and was thought to play roles in immune responses and inflammation [19,20]. Thus, there is some evidence to suggest that visfatin activates proinflammatory cytokines in human monocytes [21], as well as possibly promoting neoangiogenesis [22]. In addition, it may also exert insulin-like activity [23] leading to a direct cardioprotective effect in myocardial infarction [24], and it has also been reported to play a protective role in nonalcoholic fatty liver disease (NAFLD) [24]. Therefore, in this study we prospectively investigated plasma visfatin levels in patients with HCC and those with other clinical stages of chronic HCV or HBV infection.

2. Material and methods

2.1. Study participants

From January 2010 to August 2015, we enrolled 193 consecutive patients, of whom 57 had chronic HBV infection (38 men and 19 women; age: 50.4 ± 11.1 years), 39 had chronic HCV infection (17 men and 22 women; age: 60.1 ± 11.7 years), 22 had cirrhosis (14 men and 8 women; age: 56.5 ± 10.7 years), 36 had both HCC and chronic HBV infection (26 men and 10 women; age: 62.0 ± 10.9 years), and 39 had both HCC and chronic HCV infection (21 men and 18 women; age: 69.4 ± 9.7 years). Among the 78 patients with HCV infection, 60 had HCV genotype 1b and 18 had HCV genotype 2a. In addition, we enrolled 92 healthy adults (68 men and 24 women; age: 51.8 ± 5.9 years; body mass index (BMI): 23.8 ± 3.1 kg/m^2) without anti-HCV antibodies who were HBsAg and HIV negative, with an alcohol consumption of less than 20 g/day and normal alanine aminotransferase (ALT) level as the control groups. Chronic HBV was defined as being positivity for HBsAg (Abbott Laboratories, North Chicago, IL, USA) for at least 6 months with no evidence of HCC. Chronic HCV infection was defined as the presence of serum HCV-RNA as assayed by reverse transcription polymerase chain reaction (Amplicor Roche/Promega v.2 Diagnostic Test, Branchburg, NJ, USA). Cirrhosis was diagnosed according to child-Pugh score, and HCC was diagnosed according to histological examinations of biopsies or resected specimens. The exclusion criteria were other HCV genotypes, alcohol or drug abuse, and neoplastic, autoimmune, thyroid, and psychiatric diseases, HIV coinfection, serum ferritin level >800 ng/mL, and renal and heart failure. The study protocol and informed consent procedure were approved by the Ethics Committee of I-Shou University E-Da Hospital, and written informed consent was obtained from each participant before enrollment.

2.2. Clinical and laboratory assessments

Patients with a BMI of $\geqslant$27 kg/m^2 were classified as being obese based on the guidelines of the Department of Health, Taiwan [25]. The diagnosis of type 2 diabetes mellitus (T2DM) was made according to the World Health Organization criteria [26], with a value of fasting blood glucose level of $\geqslant$126 mg/dL on at least two occasions, or ongoing treatment with hypoglycemic agents. Metabolic syndrome was defined according to the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (NCEP-ATP III) [27], with modifications in the definition of central obesity as follows: hyperglycemia (fasting glucose $\geqslant$110 mg/dl) in addition to at least two of the following: (1) elevated arterial blood pressure $\geqslant$130/85 mmHg, (2) central obesity (waist circumference, males >90 cm; females >80 cm), (3) serum triglyceride level >150 mg/dl, and (4) serum high-density lipoprotein (HDL)-cholesterol <40 mg/dl in males or <50 mg/dl in females. Serum biochemical parameters and complete blood cell counts were also measured after overnight fasting including triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, iron, Fe, ferritin, transferrin iron binding capacity (TIBC), alkaline phosphatase (ALKP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), red cell distribution width-coefficient of variation (RDW-CV), red cell distribution width-standard deviation (RDW-SD), total bilirubin, hemoglobin, hematocrit, white blood cell count, platelets, albumin, alpha-fetoprotein (AFP), and prothrombin time, all of which were measured using standard commercial methods on a parallel, multichannel analyzer (Hitachi 7170A, Tokyo, Japan) as described in our previous report [28]. Serum insulin levels were determined by enzyme-linked immunosorbent assay (ELISA) kits (Crystal Chem; Downers Grove, USA). The intra-assay coefficient of variation was 3.5–5.8%. The homeostasis model assessment estimate of insulin resistance (HOMA-IR) was estimated using equations as previously described [29]. The concentration of plasma visfatin was determined using commercial ELISA kits (Phoenix Pharmaceuticals, Belmont, CA, USA). The intra-assay coefficient of variation was 2.4–2.7% for visfatin. Samples were measured in duplicate in a single experiment. Aspartate aminotransferase to platelet ratio index (APRI) was calculated as [(AST/UL)/platelet count ($\times 10^3$)] $\times$ 100.

2.3. Statistical analysis

Continuous, normally distributed variables are expressed as mean ± SD, and non-normally distributed variables as the median (interquartile range). The Kolmogorov-Smirnov test was used to evaluate the normality of distribution. Differences in variables between groups were tested using one-way analysis of variance (ANOVA) for normally distributed variables, followed by Tukey's pairwise comparison. Categorical data are expressed as a number (percentage), and inter-group comparisons were performed using the χ^2 test or Fisher's exact test. Logarithmic transformations were analyzed to correct for the skewed distribution of serum AST, ALT, fasting insulin, HOMA-IR index, total bilirubin, alpha-fetoprotein, and prothrombin time.