Arab Journal of Gastroenterology 17 (2016) 61–66

Contents lists available at ScienceDirect

Arab Journal of Gastroenterology

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

Original Article

The role of vascular endothelial growth factor −634 G/C and its soluble receptor on chronic liver disease and hepatocellular carcinoma

Neneng Ratnasari [a,*], Siti Nurdjanah [a], Ahmad H. Sadewa [b], Mohammad Hakimi [c]

[a] Internal Medicine Department, Faculty of Medicine, Gadjah Mada University/Dr. Sardjito General Hospital, Yogyakarta, Indonesia
[b] Biochemistry Department, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia
[c] Public Health Department, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia

ARTICLE INFO

Article history:
Received 29 June 2015
Accepted 12 June 2016

Keywords:
VEGF −634 G/C
sVEGFR-2
Chronic liver disease
Hepatocellular carcinoma

ABSTRACT

Background and study aims: The single nucleotide polymorphism (SNP) of the vascular endothelial growth factor (VEGF) gene −634 G/C (rs2010963) influences the progression of hepatocellular carcinoma (HCC). There have been no studies on the role of VEGF SNP −634 G/C in chronic liver disease (CLD). The aim of the present study was to analyse the correlation between VEGF SNP −634 and the clinical severity of CLD and HCC.

Patients and methods: A cross sectional study was conducted on 182 subjects (46 HCC, 39 liver cirrhotic/LC, 38 chronic hepatitis/CH; and 57 healthy subjects). The study was conducted from 2010 to 2014 at the Dr. Sardjito Hospital Yogyakarta, Indonesia. All subjects submitted blood serum for DNA sequencing examination using primer. The clinical data of CLD and HCC were assessed, and sVEGFR-2 was examined in 149 subjects. All data were analysed using STATA programme 11.0.

Results: Significant differences were observed in genotypic frequency (GG/GC/CC) between HCC, LC, CH and healthy subjects ($p = 0.004$), but though no significant differences were observed between the G>G and C>G genotypic frequencies ($p = 0.337$). The frequency of genotype GG was significantly higher than genotype GC or CC in HCC and was associated with declining of clinical conditions ($p < 0.05$). No significant difference in the distribution genotypes was observed with respect to the level of sVEGFR-2 in the serum. However, we observed a significant correlation between sVEGFR-2 and clinical characteristics in LC and CH ($p < 0.05$).

Conclusion: Genotype GG of the VEGF SNP −634 is the dominant genotype in severe CLD and HCC. sVEGFR-2 correlates with the disease severity but is not directly associated with the SNP −634 genotype.

Introduction

Based on the clinical course of disease, chronic hepatitis (CH) may progress to cirrhosis or liver carcinoma. However, in some patients with CH, liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC) will never develop. The poor liver progression is caused by growth factors that trigger neo-angiogenesis in response to chronic hypoxia, leading to necro-inflammation and hepatocyte cell regeneration in liver tissue. Vascular endothelial growth factor (VEGF) is one of the factors that promote angiogenesis [1–3]. Polymorphisms in the VEGF gene affects VEGF ligands and the expression of VEGF receptors [4]. The alteration of VEGF and VEGF receptors in the serum and plasma is correlated with its expression in liver tissue [5,6]. The levels of soluble VEGF receptor (sVEGFR) in the serum and plasma are similar, but the levels of VEGF in the serum differ from those in plasma [7–9]. Previous studies on HCC patients after surgery, transplantation, and chemotherapy revealed that single-nucleotide polymorphisms (SNP) at location −2578 C/A (rs699947; promoter) and −634 G/C (rs2010963; 5′-untranslated region (UTR)) are the best indicators of survival and recurrence [10,11]. In addition, the serum level of sVEGFR in serum can be analysed as a predictor of disease progression and survival after surgery and chemotherapy [12,13]. As we know by our knowledge, no previous studies focused on VEGF SNPs and serum sVEGFR as the predictors of disease progression from CH to cirrhosis and/or HCC. The aim of this study was to identify the relationship of the VEGF gene polymorphism −634 G/C with sVEGFR-2 serum levels and clinical characteristics as a predictor of the progression of chronic liver disease to HCC.

* Corresponding author at: Dr. Sardjito General Hospital, Kesehatan Street No.1 Sekip, Yogyakarta 55281, Indonesia. Fax: +62 274553120.
E-mail address: nenengratnasari@yahoo.com (N. Ratnasari).

http://dx.doi.org/10.1016/j.ajg.2016.06.005
1687-1979/

Patients and method

Study design

A cross-sectional research study with consecutive sampling was conducted from 2010 to 2014 on CH, LC, and HCC patients at the polyclinic and internal disease ward of Dr. Sardjito General Hospital in Yogyakarta, Indonesia.

Subjects

The CH patients in this study had a >6-month history of hepatitis B (HBsAg positive and IgM-anti-HBc negative) or hepatitis C (total-anti-HCV positive) infection. The LC patients were diagnosed based on clinical and laboratory symptoms, and liver imaging. The HCC patients were diagnosed based on histological examination of liver biopsy (fine-needle biopsy) or based on the noninvasive criteria for HCC diagnosis in the background of LC as follows: a lesion >2 cm with hypervascularisation after two rounds of imaging examination (liver ultrasound and computed tomography scanning) or a hypervascularised lesion >2 cm observed on one imaging examination with α-fetoprotein (AFP) levels >400 ng/ml. All subjects were >18 years of age and clinically stable. Subjects admitted as inpatients for haemorrhage in the alimentary canal were included in the study after bleeding stopped with no relapses within at least 1 week. The exclusion criteria were as follows: severe sepsis and other comorbidities, such as chronic kidney disease, heart failure, obstructive lung disease, and non-liver malignancy. All patients read the research procedure and signed the informed consent form.

Variables

The dependent variable was the presence of the VEGF gene polymorphism −634 and sVEGFR-2 serum levels. The independent variable was the clinical severity of the disease (CH, LC, and HCC). The VEGF DNA isolation and polymerase chain reaction (PCR) of the gene target −634 (rs2010963) were conducted at the Biochemistry Laboratory of Faculty Medicine, Gadjah Mada University, Indonesia. DNA sequencing (Sanger-Coulson techniques) using Applied Biosystems (ABI) chromatography was performed on target DNA with two primers ((F)5′-CCGACGGCTTGGGGA GATTGCTC-3 and (R)5′-CGGCGGTCACCCCCAAAAGCAG-3). The DNA sequencing was performed at the First Base Laboratories Sdn. Bhd., Malaysia. The serum level of sVEGFR-2 was determined using the enzyme-linked immunosorbent assay (ELISA) technique (Quantikine® HS kit, human immunoassay R&D Systems, Minneapolis, MN, USA); every measurement was performed twice.

Statistical analyses

Data were analysed using STATA 11.0. The Mann–Whitney test and Kruskal–Wallis test were used for data distribution. The receiver operating characteristic-area under curve (ROC-AUC) test was used to calculate the clinical cutoff levels for sVEGFR-2. The Data Baser V.4.16.0 software was used to read electropherograms. The target sequence was compared with the reference sequence using FASTA Server software Uva to determine the polymorphisms.

Differences in the frequency of polymorphisms were calculated using the chi-squared test or Fisher's exact test. The frequency of polymorphisms in the population was calculated using the Hardy–Weinberg Equilibrium (HWE). The predictive factors were calculated using ratio prevalence (RP) test. The correlation between variables was analysed using Spearman correlation. A p-value < 0.05 was used to assess significance.

Ethic and informed consent

This study protocol was approved by the Ethics Committee of the Medical and Health Research of the Faculty of Medicine Gadjah Mada University, based on the ethics principle outlined in the Declaration of Helsinki 2008. All subjects read and signed the informed consent form before being enrolled in the study. A permission letter for the study protocol was submitted by the director of Dr. Sardjito General Hospital, Yogyakarta, Indonesia.

Results

Characteristics of subjects

DNA serum was isolated from 182 patients and submitted for PCR to examine SNP VEGF −634 (46 HCC subjects, 39 LC subjects, 38 CH subjects, and 59 healthy subjects). The serum level of sVEGFR-2 was measured in 149 subjects (46 HCC subjects, 38 LC subjects, 38 CH subjects, and 27 healthy subjects). The characteristics of the research subjects are listed in Table 1. In this table, significant differences in age between the HCC, LC, and CH subjects relative to healthy subjects are seen. Male HCC and LC patients were more common than female patients, whereas CH female patients were more common than male patients. Hepatitis B virus (HBV) was the most common aetiology amongst the subjects, although some of the HCC and LC subjects did not have hepatitis B/C infection (HCC: 15/46; 32.6%, and LC: 10/39; 25.6%). Based on the Child–Pugh–Turcotte (CPT) score criteria, there was no significant difference between HCC and LC.

Difference in the genotypic and allelic frequencies and their association with disease severity

We observed significant difference in the genotypic frequency of the VEGF SNP −634. The genotype GC (73.63%) was more frequent than GG (23.08%) or CC (3.29%). Significant differences were observed in the frequencies between healthy subject and patients with HCC ($p = 0.002$) and CH ($p = 0.003$). Based on the HWE, the G allele was more common than the C allele, and significant differences were observed in allelic frequency between healthy subjects and HCC ($p = 0.047$) and CH ($p = 0.036$) patients (Table 2).

The relationship between genotypic frequency and clinical characteristics was significant only in HCC subjects. The frequency of the GG genotype was more common in patients exhibiting poor clinical criteria than those of the GC and CC genotypes ($p = 0.043$ for the CPT criteria, $p = 0.033$ for the Okuda criteria, and $p = 0.014$ for the Barcelona Clinic Liver Cancer Group (BCLC) criteria). Based on an analysis of its odd ratio (OR) >1, the polymorphism G/C may represent a predictive factor of diseases. The prevalence of HCC could be predicted based on the detection of G/C in CH patients (OR 1.555) relative to healthy subjects (OR 2.232). The prevalence of LC could be predicted based on the detection of G/C in CH patients (OR 1.519) relative to healthy subjects (OR 2.18). The prevalence of CH could be predicted in healthy subjects (OR 1.434).

Relationship between sVEGFR-2 levels, VEGF SNP G/C, and clinical criteria

The median serum sVEGFR-2 level differed significantly amongst the subjects. The median level of sVEGFR-2 was found to be the least in LC subjects (7014.95 pg/mL) and highest in healthy subjects (9785.2 pg/mL) (Table 3). Based on the post hoc analysis, significant differences in the sVEGFR-2 levels were detected amongst all groups of subjects (HCC–LC $p = 0.006$; HCC–CH $p = 0.212$; HCC–healthy $p = 0.0007$; LC–CH $p = 0.0001$; LC–