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Serum *Wisteria floribunda* Agglutinin Positive Mac-2-Binding Protein and Fib-4 Index on the Clinical Course of Patients with Chronic Hepatitis C Receiving Daclatasvir/Asunaprevir Therapy

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Abstract

Background/Aim: Serum *Wisteria floribunda* agglutinin positive Mac-2-Binding Protein (M2BPGi) is a noninvasive glycolytion-marker for fibrosis. In this study, we analyzed serial changes of fibrous markers during the clinical course of patients with chronic hepatitis C receiving daclatasvir/asunaprevir therapy.

Methods: A total 31 chronic hepatitis C and cirrhotic patients recruited from September 2014 to February 2017 were treated with daclatasvir/asunaprevir therapy for 24 weeks. Serum M2BPGi were measured prior to, at the End of Treatment (EOT) and at 24 weeks after the completion of treatment. We also measured Alpha-Fetoprotein (AFP) levels, serum albumin levels and the Fibrosis-4 (Fib 4) index during the therapy and follow-up period.

Results: Thirty patients (96.7%) achieved Sustained Viral Response (SVR). AFP levels decreased significantly. Serum albumin levels improved significantly during the clinical course. M2BPGi levels and Fib-4 index continuously improved significantly.

Conclusion: Daclatasvir/asunaprevir therapy achieves high SVR rate and improve fibrosis markers such as M2BPGi and Fib-4 index.

Keywords: ***Wisteria floribunda*** **agglutinin positive Mac-2-binding protein; Daclatasvir; Asunaprevir; FIB-4 index**

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Introduction

There are estimated 180 million Hepatitis C Viruses (HCV) infected patients worldwide [1,2]. It is estimated that 15% to 30% of such patients will develop serious complications, including liver cirrhosis, end-stage liver disease and hepatocellular carcinoma [3]. Therapeutic goals for chronic hepatitis C are negative levels of Hepatitis C Virus-Ribonucleic Acid (HCV-RNA), low levels of Alanine Aminotransferase (ALT), and ultimately inhibition of hepatocarcinogenesis and chronic liver failure by maintaining undetectable levels of HCV-RNA. In September 2014, the National Health Insurance system in Japan approved the use of the Interferon (IFN) free Direct-Acting Antiviral (DAA) agent Daclatasvir (DCV) in combination with Asunaprevir (ASV) as dual therapy against genotype 1 chronic hepatitis C [4]. This regimenis orally administered with minimal side effects, making it easier to use in patients who are at high risk of developing hepatocellular carcinoma, such as patients with hepatic fibrosis and elderly patients. This therapy has shown high Sustained Viral Response Rates (SVR).

Recently, the advancement in glycol-chain biology has enabled the measurement of *Wisteria floribunda* agglutinin-positive Mac-2 Binding Protein (M2BPGi), an isoform of the glycan structure of M2BP. Serum M2BPGi levels have been correlated with increasing severity of liver fibrosis in patients with chronic hepatitis [5]. The present study investigated the liver fibrosis markers such as M2BPGi and FIB-4 index as a tool for monitoring lowering hepatocarcinogenesis risk in chronic hepatitis type C during DCV/ASV therapy.

Methods

A prospective observational design was used to conduct this study. A total 56 chronic hepatitis

Table 1: Patient characteristics.

	n=31
Age (years)	69.6 ± 10.6
Sex (male/female)	16/15
Chronic hepatitis/Liver cirrhosis	25/6
HCV RNA (log IU/ml)	5.9 ± 0.9
Drug resistance mutation	NS5A 1/NS3 3/both 0
Previous treatment history	Non 14/IFN 3/PEGIFN+RBV 14
Albumin (g/dl)	3.8 ± 0.5
ALT (IU/l)	39.9 ± 23.2
Platelet count (× 10^4/μ)	13.3 ± 8.1
AFP (ng/ml)	18.4 ± 3.4

ALT: Alanine Aminotransferase; AFP: α-fetoprotein

C and cirrhotic patients underwent daclatasvir/asunaprevir therapy were invited to participate in the study from September 2014 to February 2017 at Saiseikai Niigata Daini Hospital (Niigata, Japan). Written informed consent was obtained from all patients, and the Ethical Committee of Saiseikai Niigata Daini Hospital (Niigata, Japan) approved this study, which was conducted in accordance with the Declaration of Helsinki. All patients received fixed dose of daclatasvir (60 mg once daily) and asunaprevir (100 mg twice daily) for 24 weeks. M2BPGi quantification was performed by a WFA-antibody immunoassay using a commercially available kit (HISCL M2BPGi; Sysmex Co., Kobe, Japan) and a fully automatic immunoanalyzer (HISCL-5000; Sysmex Co.). HCV-RNA were measured using the Real Time HCV assay (Abbott Park, IL, USA) with a Lower Limit Of Qualification (LLOQ) of 12 IU/mL at baseline, every 2 weeks during treatment and every 2 weeks until 24 weeks after completion or cession of the dual oral therapy. Sustained Viral Response (SVR) was defined as negative for serum HCV RNA at 24 weeks after End-of-Treatment (EOT). FIB-4 index includes four factors: age, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and platelet counts [6].

Thirty-one patients of a total of 56 patients met the following inclusion criteria: 1) Diagnosis of chronic hepatitis C; 2) Negativity for hepatitis B surface antigen or human immunodeficiency virus; 3) Negative history of other chronic liver diseases (autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease); 4) Absence of Hepatocellular Carcinoma (HCC) or any suspicious lesions detected through ultrasonography, dynamic computed tomography, or magnetic resonance imaging at enrollment; 5) Negative history of previous treatment for HCC and liver transplantation; 6) A follow-up period of 0.5 year after the EOT; 7) Absence of HCC development at 24 weeks after the EOT. Patient characteristics, biochemical data, hematological data, virological data and treatment details were collected at enrollment. To evaluate albumin, Fib-4 index, Mac-2-Binding Protein (M2BPGi), serum α-Fetoprotein (AFP) was collected at baseline and during follow up.

Statistical analysis

Continuous variables were analyzed using the Mann-Whitney U-test, and categorical variables were analyzed using the χ^2-test. Values of $P<0.05$ were considered statistically significant. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), is a modified version R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics [7].

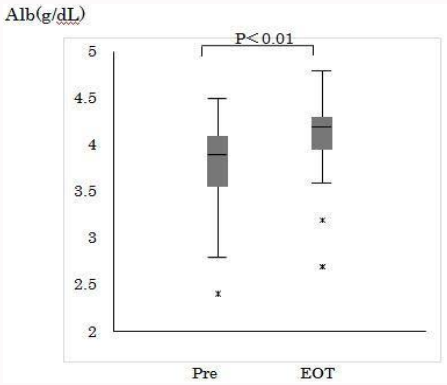

Figure 1: Sequential changes in serum albumin levels. In these box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 75th and 25th percentiles, respectively; the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.
Alb: Albumin; Pre: Pretreatment; EOT: End-of-Treatment.

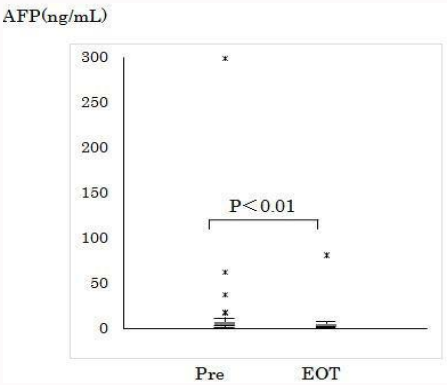

Figure 2: Sequential changes of serum alpha-fetoprotein. In these box-and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 75th and 25th percentiles, respectively; the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.
AFP: Alpha-Fetoprotein; Pre: Pretreatment; EOT: End-of-Treatment.

Results

A total of 31 patients with a mean age of 69.6 years participated in this study (Table 1). Of these patients, 10 (32.3%) were elderly individuals aged 75 years and above. There were 6 patients (19.4%) with liver cirrhosis, one patient (3.2%) had drug resistance mutations in Nonstructural Protein 5A (NS5A) and 3 patients (9.7%) had Nonstructural Protein 3 (NS3) drug resistance variants. Among all, thirteen patients (41.9%) had no previous treatment history. Fourteen patients (45.2%) received Pegylated IFN and Ribavirin (PegIFN/RBV). Based on Intent-to-Treat (ITT) analysis, negative HCV-RNA rates were as follows: 4W 82.8%, 8W 100%, 12W 100%, 16W 100%, 20W 100%, 24W 100%, and SVR24 96.8%. In the non-mutant patients, 100% had SVR24, 100% had liver cirrhosis, and 96% had chronic hepatitis. While there were no unresponsive patients, there was one case of recurrence. This patient had previous treatment histories of receiving PegIFN/RBV, and D168 variant and IL-28 heterotype was observed at baseline.

The incidence of adverse events was 22.6%. Three patients experienced liver dysfunction, and 3 patients developed

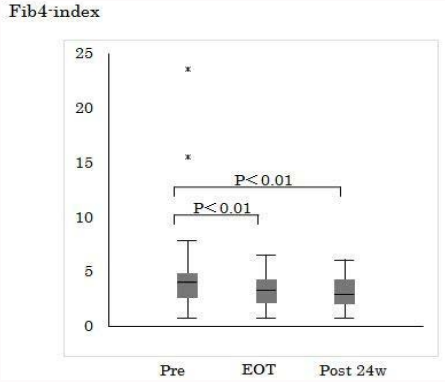

Figure 3: Sequential changes in Fib-4 index. In these box and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 75th and 25th percentiles, respectively; the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.
Pre: Pretreatment; EOT: End-of-Treatment; Post 24W: 24 Weeks after End-of-Treatment.

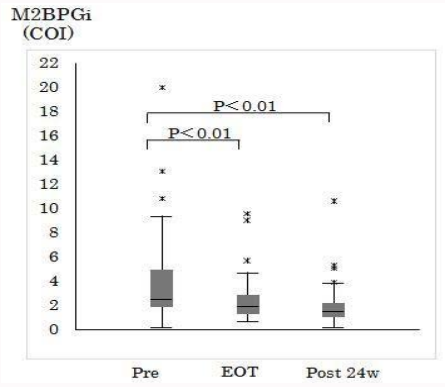

Figure 4: Sequential changes in M2BPGi. In these box and-whisker plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 75th and 25th percentiles, respectively; the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively.
M2BPGi: Mac-2-Binding Protein; Pre: Pretreatment; EOT: End-of-Treatment; Post 24W: 24 Weeks after End-of-Treatment.

dermatological symptoms such as rash and pruritus. No patient discontinued treatment, liver dysfunction was observed in one patient. Serum Albumin (Alb) improved from 3.9 g/dl to 4.2 g/dl (Figure 1). AFP levels before treatment were 18.4 ng/ml and decreased to 6.1 ng/ml (Figure 2). FIB-4 index also improved continuously from 5.0 to 3.4 and then to 3.1 (Figure 3). Similarly, M2BPGi levels also continuously improved as follows: i) 3.8 before treatment; ii) 2.6 at EOT; and iii) 2.1 at SVR24 (Figure 4).

Discussion

Chronic Hepatitis C Virus (HCV) infection frequently causes liver cirrhosis and Hepatocellular Carcinoma (HCC) development. HCC is currently one of the most common cancers and cause of cancer-related death worldwide [8]. The treatment goal for chronic hepatitis C is to prevent progression of hepatic fibrosis and to inhibit the development of HCC. In order to achieve this goal, complete eradication of HCV is essential. In July 2014, the first IFN-free drug regimen, asunaprevir and daclatasvir combination therapy was approved in Japan. The phase III clinical trials for this treatment in Japan enrolled 222 patients with genotype 1 chronic hepatitis C, and the criteria included elderly patients up to age 75 with Child-Pugh A compensated cirrhosis. The SVR rates for patients aged below 65 and aged 65 and above were 81.2% (108/133) and 89.9% (80/89), respectively. Similarly, the SVR rates for non-cirrhosis patients and compensated cirrhosis patients were 84.0% (168/200) and 90.9% (20/22), respectively. High levels of SVR were achieved, regardless of age and stage of hepatic fibrosis. In addition, an SVR rate of 80.5% (70/87) was achieved among patients who were unresponsive to previous treatments [4]. This study has demonstrated that patients who have drug resistant variants in the NS3 and NS5A regions prior to treatment experience reduced therapeutic effect of asunaprevir and daclatasvir combination therapy. IFN-free DAA are widely used in patients with hepatic fibrosis, a group known to be at extremely high risk for hepatocellular carcinoma. However, there have been no previous studies that have examined the risk of developing HCC among SVR patients who received IFN-free DAA treatment.

Recently, a new glycol marker for liver fibrosis was developed using the glycan sugar chain-based immunoassay. *Wisteria floribunda* agglutinin-positive Mac-2-Binding Protein (M2BPGi) was identified as a fibrosis-related glycol-alteration [5] and a significant association between its serum levels and histological hepatic fibrosis was reported in chronic liver diseases [9]. Sasaki et al. [10] have reported that M2BPGi is an effective HCC prognostic tool after HCV eradication. This suggests that HCC screening might be essential after viral eradication if M2BPGi levels do not sufficiently decline at the time of HCV eradication. In the present study, we compared the value of M2BPGi in serum during DCV/ASV therapy in hepatitis C patients. In this study, 96.7% achieved SVR 24, a favorable SVR rate. When patients with resistance variants were excluded at baseline, SVR 24 was extremely high at 96.2%. The incidence of adverse events was 22.6% and primarily included rash and liver dysfunction. The treatment improved hepatic markers such as albumin, M2BPGi, and the Fib4-index, which are used to assess liver function. Hence, M2BPGi could not always predict early cirrhosis in non-viral cirrhosis [11]. M2BPGi reflects hepatic fibrosis as well as hepatic inflammation. In conclusion, liver function parameters were improved and liver fibrosis markers reduced in chronic hepatitis C patients who achieved SVR following daclatasvir and asunaprevir combination treatment. These results suggest that DCV/ASV treatment has the potential to improve liver fibrosis and decrease the incidence of hepatocarcinogenesis in chronic hepatitis C patients.

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