The role of serum Wisteria floribunda agglutinin-positive Mac-2 binding protein in the assessment of fibrosis in children with chronic hepatitis C

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Abstract

Background

At present non-invasive fibrosis markers are not available for assessment of liver fibrosis in children with chronic hepatitis C.

Methods

Sixty-three children with chronic hepatitis C were included. Changes in *Wisteria floribunda* agglutinin-positive Mac-2 binding protein (M2BPGi) levels were evaluated in 13 of 27 treatment-naïve patients during the observation period of the natural course of disease (median 4, range 3-6 years). Changes during treatment were evaluated in 27 of 36 patients for 4 (2-9) years of post-treatment follow-up.

Results

There were significant differences in the levels of M2BPGi between the control group and HCV F0 group ($P=0.002$) and between the control group and HCV F1 group ($P<0.001$). Receiver operating characteristic curve analysis showed that to detect stage F1 fibrosis cut off value was 0.95 for M2BPGi with sensitivity 52%, specificity 90%, and area under the curve 0.687. A substantial decrease in M2BPGi levels by treatment was shown from 0.98 ± 0.57 at pre-treatment to 0.42 ± 0.15 at post-treatment ($P<0.001$) in the 27 treated-patients.

Conclusions

Our study shows new findings that M2BPGi may be useful to predict the presence of a mild degree of fibrosis in children with chronic hepatitis C and such a mild fibrosis may be quickly resolved by antiviral therapies.

Introduction

Recently, various noninvasive methods to evaluate liver fibrosis have been developed, including APRI, biomarkers, and elastography using ultrasonography (1–3). Although elastography has a high diagnostic ability for liver fibrosis and is widely used, it has several drawbacks, such as the requirement for expensive equipment, limited available facilities, and equipment incompatibility. Blood biomarkers are also widely used to evaluate liver fibrosis and prognosis in patients with chronic hepatitis (4–6). Recent advances in non-invasive assessment of liver fibrosis based on serum biomarkers and imaging have been summarized in reviews (4) (7).

Wisteria floribunda agglutinin-positive Mac-2 binding protein (M2BPGi) is a recently recognized liver fibrosis biomarker that was developed as a marker for HCV infection in Japan (8) (9). M2BPGi is a N-glycosylated glycoprotein and is secreted as a ligand of galectin-3 (Mac-2). Many studies have compared the diagnostic accuracy of M2BPGi with that of liver biopsy in identifying liver fibrosis stage in adult

patients. In all cases, M2BPGi levels significantly increased as liver fibrosis progressed, which confirmed the utility of M2BPGi for diagnosing liver fibrosis in chronic hepatitis C (10) (11). M2BPGi is now widely used for diagnosing liver fibrosis in chronic liver diseases because it can be easily measured in the serum (9) (12) (13). Furthermore, an increase in M2BPGi levels over time has been associated with HCC risk (13) (14) (15). A meta-analysis confirmed the utility of M2BPGi in diagnosing fibrosis and predicting HCC risk (15).

At present a liver biopsy is the only reliable method in evaluating liver fibrosis in children (16) (17). No reliable fibrosis markers are available for assessment of liver fibrosis in children with chronic hepatitis C. In the present study we measured serum M2BPGi in children with chronic hepatitis C at both pre- and post-treatment and found that this non-invasive marker is a useful tool for evaluation of liver fibrosis in such patients.

Patients And Methods

Control children for reference levels

We determined reference levels of M2BPGi in 104 children without any kind of liver disease. The control subjects consisted of 68 boys and 36 girls with a median age of 10.5 years (range 4-17). Among those children platelet counts and serum levels of AST and ALT were assayed and confirmed to be within normal ranges on the same day that serum was collected for an M2BPGi assay. Sera were stored at -20°C until assayed. Written informed consents were obtained from a parent and/or legal guardian for study participation of the control children group as well as chronic hepatitis C children group.

Reproducibility of M2BPGi measurements

To evaluate the reproducibility of M2BPGi measurements, the marker was assayed twice within a median of three months (range, 2-6) in 26 children who visited one of six facilities every three months and whose underlying liver diseases were regarded to be clinically cured or to remain stable in remission. The group consisted of 12 with a prolonged sustained virologic response (SVR) after treatment for chronic hepatitis C, 10 with HBV infection in an inactive carrier stage, two with autoimmune hepatitis in a sustained remission, and two with fatty liver in a stable condition. Some data in the group of chronic hepatitis C were utilized for post-treatment measurements as described below.

Children with chronic hepatitis C

Sixty-three children with chronic hepatitis C were included for this study, who visited one of six participating institutions between January 1996 and November 2012. Thirty-six patients underwent anti-viral therapies, and 27 patients were treatment-naïve. Changes in M2BPGi during the observation period of the natural course of disease were evaluated in 13 of the 27 children with chronic hepatitis C. Changes during treatment were evaluated in 27 of the 36 children who underwent therapy for chronic hepatitis C, all of whom responded to treatment with an SVR. Treatment regimens included combinations of peg-

interferon and ribavirin (n=19), simeprevir plus peg-interferon and ribavirin (n=3), ledipasvir and sofosbuvir (n=3), and ribavirin and sofosbuvir (n=2).

This study was approved by the ethics committee of Osaka General Medical Center. Participation in the study was posted at each participating institution. The study protocol conforms with the ethical guidelines of the Helsinki Declaration.

Measurement of hepatitis viral markers

Anti-HCV antibodies were determined by ELISA (Abbott, Chicago, IL, USA). HCV RNA was measured by RT-PCR (COBAS AMPLICOR HCV MONITOR assay version 2.0, Roche Diagnostics, Tokyo, Japan). HCV serotyping was determined by detecting antibodies against group-specific recombinant proteins for serotypes 1 and 2 in the putative HCV NS4 protein region by an enzyme immunoassay. HCV genotyping was performed according to the international classification (18).

Measurement of fibrosis markers

M2BPGi measurements were automated using the HISCL-2000 instrument (Sysmex Co., Hyogo, Japan) (6, 9). The measured M2BPGi were indexed with the obtained levels using the following equation: Cut-off index (COI) = ([M2BPGi]sample - [M2BPGi]NC)/([M2BPGi]PC - [M2BPGi]NC), where the [M2BPGi] sample is the M2BPGi count of the serum sample, and PC and NC are the positive and negative controls. The positive control was a calibration solution preliminarily standardized to yield a COI value of 1.0.

The AST to platelet ratio index (APRI) was calculated using the following equation: $APRI = ((AST / \text{upper limit of normal range of AST}) \times 100) / \text{platelet count } (10^9/L)$. FIB-4 was calculated according to the following equation: $FIB-4 \text{ index} = (\text{age [years]} \times AST [IU/L]) / (\text{platelet count } [10^9/L] \times (ALT [IU/L])^{1/2})$. We calculated the APRI index and FIB-4 index by using the data of age, values of AST and ALT, and platelet count on the same day when serum was collected for the M2BPGi assay.

Histopathology of the liver

Histopathology of the liver was evaluated using the first liver biopsy specimens from children with chronic hepatitis C who were treatment-naïve. Liver biopsy specimens were assessed pathologically based on the New Inuyama Classification of chronic hepatitis (19). In this classification, chronic hepatic diseases are classified according to the degree of fibrosis (F) as follows; F0 (no fibrosis, the same degree as Ishak stage 0), F1 (fibrosis portal expansion, the same degree as Ishak stage 1-2), F2 (bridging fibrosis, the same degree as Ishak stage 3), F3 (bridging fibrosis with lobular distortion, the same degree as Ishak stage 4), and F4 (cirrhosis, the same degree as Ishak stage 5-6) (19), (20). Additionally, chronic hepatic diseases are classified based on the degree of inflammation and necrosis of hepatocytes. The activities (A) are classified as follows; A0 (no necro-inflammatory reaction), A1 (mild necro-inflammatory reaction), A2 (moderate necro-inflammatory reaction), and A3 (severe necro-inflammatory reaction) (19).

Statistical analysis

Data were analyzed with the chi-square test, Student's t-test and Mann-Whitney's U-test. P value <0.05 was considered statistically significant. Statistical analyses and the receiver operating characteristic curve (ROC) analysis were performed using JMP Pro 12 (SAS Institute Inc., Cary, NC, USA).

Results

Subject's characteristics

The baseline characteristics of children with chronic hepatitis C and control children are shown in Table 1. Circulating platelet counts were significantly lower in the HCV group than in the control group as well as were the FIB-4 index scores. Both serum AST and ALT levels were significantly higher in the HCV group than in the control group as well as were the APRI index scores and M2BPGi levels.

Thirty-three children with chronic hepatitis C underwent a liver biopsy in this study. No active necro-inflammation was observed in three (9%) patients, while mild (A1) and moderate activities (A2) were observed in 27 (82%) and three (9%) patients respectively. No severe activity (A3) was observed. In terms of liver fibrosis F0 was noted in nine (27%) patients, and mild (F1) and moderate degrees of liver fibrosis (F2) were observed in 22 (67%) and two (6%), respectively. Advanced liver fibrosis (F3 or F4) was not found among the present children (Table 1).

Reference values in control children

M2BPGi levels from 100 of the 104 control children were below 1.0. The remaining four children showed a level greater than 1.0 at first examination. Repeated assays performed a few months later (3-4 months) showed a level less than 1.0 in all the four subjects. After excluding those four children, the mean value with standard deviation for 100 control children was 0.57 ± 0.19 (Figure 1).

Reproducibility

Levels of M2BPGi at the first and second assay were 0.42 ± 0.15 and 0.47 ± 0.15 , respectively. Differences between the two assays were 0.13 ± 0.08 in 26 children whose underlying liver diseases were clinically cured or stable in remission (Supplementary Figure 1). Accordingly, a change in M2BPGi of greater than 0.30 COI was arbitrarily regarded as significant in this study because the average plus $2.0 \times \text{SD}$; $0.13 + (0.08 \times 2.0) = 0.29$.

Serum M2BPGi levels in children with chronic hepatitis C

There was a marginal but significant correlation between M2BPGi and APRI ($r=0.332$, $P=0.007$, $n=46$), but not between M2BPGi and FIB-4 index ($r=0.143$, $P=0.262$, $n=46$) (data not shown). The serum M2BPGi levels of HCV-infected children were greater than those of control subjects (0.95 ± 0.55 vs. 0.57 ± 0.19 , $P=0.001$) (Table 1).

There was a slight but significant difference in the levels of M2BPGi between the control group and the HCV F0 group ($P=0.002$, Figure 1). The levels of M2BPGi in the HCV F1 group were also significantly higher than those in the control group ($P<0.001$), but not for the HCV F0 group ($P=0.186$, Figure 1). Because only two patients showed F2 fibrosis no comparison was made between the F2 group and any other groups.

ROC analysis

ROC analyses showed that to detect stage F1 fibrosis the cut off value was 0.95 for M2BPGi, with a sensitivity of 52%, specificity of 90%, and area under the curve (AUROC) 0.687 (Figure 2A). The cut off value was 0.37 for APRI with a sensitivity of 73%, specificity of 70%, and AUROC 0.639 (Figure 2B). The cut off value was 0.22 for FIB-4 with a sensitivity of 78%, specificity of 60%, and AUROC 0.680 (Figure 2C). Our results suggest that M2BPGi as well as APRI and FIB-4 are good markers for predicting the presence of hepatic fibrosis in children with chronic hepatitis C.

Changes in serum M2BPGi levels in children with chronic hepatitis C

Changes in M2BPGi levels during the observation period of the natural course of disease were evaluated in 13 children with chronic hepatitis C. The median duration from the first assay to the second assay was four years (range, 3-6) (Supplementary Figure 2A). There was no significant change in M2BPGi levels from 0.66 ± 0.33 at the first assay to 0.89 ± 0.42 at the second assay ($P=0.149$, Supplementary Figure 2B). Five of the 13 showed a significant change of greater than 0.3 COI. In the remaining eight no significant changes in M2BPGi levels were observed.

Changes in M2BPGi levels during treatment were evaluated in 27 patients, all of whom showed an SVR to treatment. The median duration of the follow-up after the end of treatment was 4 years (range, 2-9) (Figure 3A). There was a substantial decrease in M2BPGi levels from 0.89 ± 0.57 at pre-treatment to 0.42 ± 0.15 at post-treatment ($P<0.001$, Figure 3B). Seventeen of the 27 showed a significant change of greater than 0.3 COI with decrement from 1.09 ± 0.61 at pre-treatment to 0.42 ± 0.16 at post-treatment ($P<0.001$, data not shown).

Discussion

The previous studies on serum biomarkers including APRI, FIB-4, and FibroTest have shown that they are useful for selecting pediatric patients with liver cirrhosis who should undergo a liver biopsy (21–24). However, chronic hepatitis C generally progresses slowly during childhood with no cases showing liver cirrhosis in our subjects, consistent with reports in previous studies (25, 26). Whether chronic hepatitis C can lead to a substantial degree of liver fibrosis may be more crucial for a pediatric population. In this regard the present study has suggested that M2BPGi, one of the non-invasive serum markers, may be useful to predict the presence of a mild degree of fibrosis in children with chronic hepatitis C and such fibrosis may be quickly resolved by antiviral therapies.

First, we examined the fundamental characteristics of M2BPGi including reference levels in the control children and reproducibility among various types of pediatric chronic liver diseases. Most of the control children showed levels below 1.0 COI which is regarded as a cut-off point for adults. Four of our control subjects showed M2BPGi levels over 1.0 COI and physical examinations done at that time revealed no symptoms or signs of liver dysfunction, infection, or inflammation. Moreover, repeated assays performed 3-4 months apart in those subjects showed levels below 1.0 COI. The reason for the apparently transient rise in M2BPGi levels is not known at present. But the marker should be examined repeatedly for reliable evaluation of liver fibrosis.

Next, this study suggests that M2BPGi may be more useful for predicting the presence of a mild degree of fibrosis (F1) than the fibrosis markers or tests that were already reported. In fact, serum M2BPGi levels from HCV-infected children with F0 or F1 liver fibrosis showed higher levels than the reference levels in control children with no liver disease. The reason for a small elevation in M2BPGi for the F0 group might indicate the presence of minute fibrosis due to a sustained necro-inflammatory reaction that is generated by chronic HCV infection. In addition, comparable performance of M2BPGi with APRI and FIB-4 was suggested by ROC analysis. Among the reviewed children with chronic hepatitis C, levels of M2BPGi were found to be elevated during the natural course of disease in many of them. Levels of M2BPGi were substantially decreased after treatment in most children who underwent antiviral treatment; including combination therapy of peg-interferon and ribavirin and direct-acting antiviral agents.

A literature survey has shown there have been no reports regarding the role of M2BPGi in children with chronic hepatitis C. There have been some reports on the evaluation of hepatic fibrosis markers associated with chronic hepatitis C; hyaluronic acid, type 4 collagen, APRI, and FIB-4 (21–23). Among them APRI and FIB-4 are regarded as a relatively reliable index of fibrosis. Our study indicates that in children, M2BPGi showed comparably good performance with APRI and FIB-4.

A limitation of this study is that no HCV-infected patients with advanced liver fibrosis (F3 or F4) were present among our subjects because chronic hepatitis C generally progresses slowly during childhood (25, 26). Another limitation is that this study was not a prospective study and examination of M2BPGi was not done in some patients during the observation period of the natural course of disease as well as in some patients during treatment.

In conclusions, our study shows that M2BPGi may be useful in evaluating early generation of liver fibrosis in children with chronic hepatitis C as well as for following its resolution after successful treatment. Our study suggests that persistent HCV infection may produce a mild degree of fibrosis during childhood and such an early pathological change may resolve after antiviral treatment.

Abbreviations

HCV, Hepatitis C virus; anti-HCV, anti-HCV antibody; SVR, Sustained virologic response; IFN, interferon; RBV, ribavirin; CHC, chronic hepatitis C; SD, standard deviation; DAAs, direct-acting antiviral agents.

Declarations

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Author's contributions: TH and SM for conception and design of the study. TH, TT, MY, IY, HR, and MJ for acquisition of laboratory and clinical data. TH, SM, BK and BS for drafting of article and critical revision. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, Table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Figure 1

M2BPGi levels were expressed by using box-and-whisker plot for control children and for three groups of patients with chronic hepatitis C (F0, F1, and F2). Mean values with a standard deviation were described for the control, F0 and F1 groups.

Figure 2A

Figure 2B

Figure 2C

Figure 2

ROC curve analysis of M2BPGi (Fig. 2A), APRI (Fig. 2B) and FIB-4 (Fig. 2C) to detect stage F1 fibrosis.

Figure 3

Change in levels of M2BPGi by treatment. In Fig. 3A, dots indicating M2BPGi levels were plotted at age in years when measurement of M2BPGi was done. M2BPGi dots at pretreatment and at post-treatment were connected with for each child both in Figures 3A and 3B.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1.Baselinecharacteristicsofthestudypatients.xlsx](#)
- [SupplementaryFigures1and2.pdf](#)