Gastroenterologia Japonica
Vol. 20, No. 3

Printed in Japan

—Original Article—

CLINICAL APPLICATION OF THE MEASUREMENT OF SERUM ASIALOGLYCOPROTEINS TO ESTIMATE RESIDUAL LIVER FUNCTION IN PATIENTS WITH CHRONIC LIVER DISEASES WITH OR WITHOUT HEPATOCELLULAR CARCINOMA

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Summary

The correlation between the amount of asialoglycoproteins and results of conventional liver function tests was studied in patients with chronic liver diseases, with or without hepatocellular carcinoma. The objective was to determine the clinical significance of the measurement of levels of serum asialoglycoproteins. The levels were elevated in accordance with the progress of liver diseases, and correlated with the decrease in albumin content, cholinesterase activity, the ratio of esterified cholesterol to total cholesterol and to the increase of indocyanine green retention at 15 min ($p<0.001$). There was no correlation with values of glutamic oxaloacetic and pyruvic transaminases. The amount of serum asialoglycoproteins also correlated with survival time in fatal cases of cirrhosis and/or hepatocellular carcinoma.

Bilirubin and bile acids did not interfere with the measurement of serum asialoglycoproteins in cases of hyperbilirubinemia.

Serum asialoglycoprotein levels are a good indicator of hepatic functional reserve in patients with chronic liver diseases, with or without hepatocellular carcinoma.

Key Words: *Serum asialoglycoproteins, Liver function tests, Hepatic functional reserve.*

Introduction

To assess prognosis, it is important to estimate hepatic functional reserve in patients with cirrhosis and/or hepatocellular carcinoma. Many tests based on the metabolism of protein, lipid, carbohydrate, bile constituents and drugs in the liver have been used for clinical assessment of residual liver function.

The survival of numerous glycoproteins in the circulation depends on the integrity of car-

Received November 15, 1984. Accepted March 11, 1985.

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We thank M. Ohara, Kyushu University for critical reading of the manuscript and Y. Tsuda for secretarial services. This work was supported in part by a grant-in-aid for scientific research and for Special Project Research from the Ministry of Education, Science and Culture, Japan.

bohydrate chains whereas removal of sialic acid residues hastens the disappearance of serum glycoproteins from the circulation to the liver. The asialoglycoprotein receptor which recognizes non-reducing terminal galactose and N-acetylgalactosamine residues of carbohydrate chains is present in rabbit, rat and human livers and is localized exclusively in hepatocytes[1,2]. Asialoglycoproteins have been reported to markedly accumulate in sera of patients with cirrhosis and/or hepatocellular carcinoma. Structural damage to hepatocytes or plasma membrane might interfer with the hepatic uptake of circulating asialoglycoproteins and lead to their accumulation in serum[3-8]. We found that the hyperasialoglycoproteinemia in galactosamine-treated rats was accompanied by a decrease in receptors and that the clearance rate of asialoorosomucoid was in inverse proportion to the receptor level[9]. Recently, we showed that the asialoglycoprotein receptor level in the cirrhotic liver decreased to one third of that in control tissues and that hepatocellular carcinoma and metastatic tumor tissues did not possess receptor activity. The amount of serum asialoglycoproteins strongly correlated with the tumor size in patients with hepatocellular carcinoma[10]. Thus, levels of asialoglycoproteins seem to depend on the amount of the receptor and the receptor level parallels the hepatic functional mass. In the present work, we determined serum asialoglycoprotein levels and carried out conventional liver function tests in patients with chronic liver diseases, with or without hepatocellular carcinoma.

Materials and Methods

Serum Samples and Diagnostic Criteria

Serum samples were collected from 108 Japanese patients with chronic liver disease, with or without hepatocellular carcinoma, and from 13 control subjects with no evidence of diseases. In some patients, several samples were taken during the course of their illness. All sera were frozen and stored at −20°C until the measurement of asialoglycoproteins. All patients had been admitted to Kansai Medical University Hospital and diagnosis had been made on the basis of signs and symptoms, clinical laboratory tests, and the histology of liver specimens obtained by biopsy and/or autopsy. Chronic hepatitis was divided into active and inactive stages, according to the histological criteria of the Japan Society of Hepatology[11]. The diagnosis of compensated and decompensated cirrhosis was based on the presence or absence of ascitis, jaundice and hepatic encephalopathy[12].

Proteins and Receptor Assay

Human orosomucoid, asialoorosomucoid and ^{125}I-asialoorosomucoid were prepared as described previously[13]. The purification of

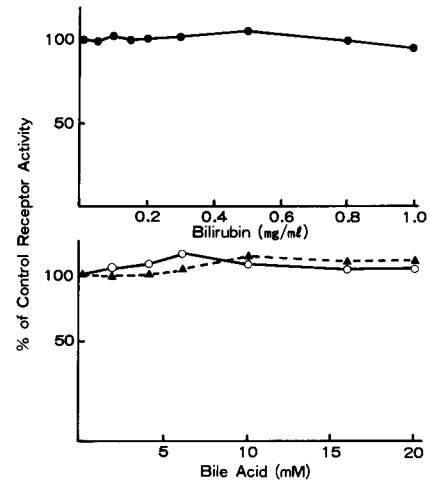

Fig. 1. Effect of bilirubin and bile acids on the receptor assay. Bilirubin was dissolved in bovine serum albumin solution. Rabbit asialoglycoprotein receptor (4 ng) was preincubated for 15 min at 25°C with various amounts of bilirubin, cholic acid and taurocholic acid. ^{125}I-asialoorosomucoid was added and the receptor assay was carried out as described in Materials and Methods.
●—● bilirubin, ▲---▲ cholic acid, ○---○ taurocholic acid

rabbit asialoglycoprotein receptor and the receptor assay were carried out according to the method of Hudgin et al.[14].

Determination of Serum Asialoglycoproteins

Serum samples were dialyzed against 0.01 M Tris-HCl buffer pH 7.8, to remove competitive inhibitors of receptor assay, including glucose. The amount of serum asialoglycoproteins was determined as described previously[9] and expressed in terms of micrograms of asialoorosomucoid required to cause equivalent inhibition of the receptor activity.

Effect of Bilirubin and Bile Acids on the Receptor Assay

Bilirubin and bile acids accumulate in sera of patients with hepatobiliary diseases. The effects of bilirubin and bile acids on receptor assay was examined. Bilirubin was dissolved in bovine serum albumin according to the method of Doumas et al.[15]. Rabbit asialoglycoprotein receptor (4 ng) was preincubated for 15 min at 25°C with various amounts of bilirubin, cholic acid or taurocholic acid. ^{125}I-asialoorosomucoid was then added and the receptor assay was carried out as described above. As shown in

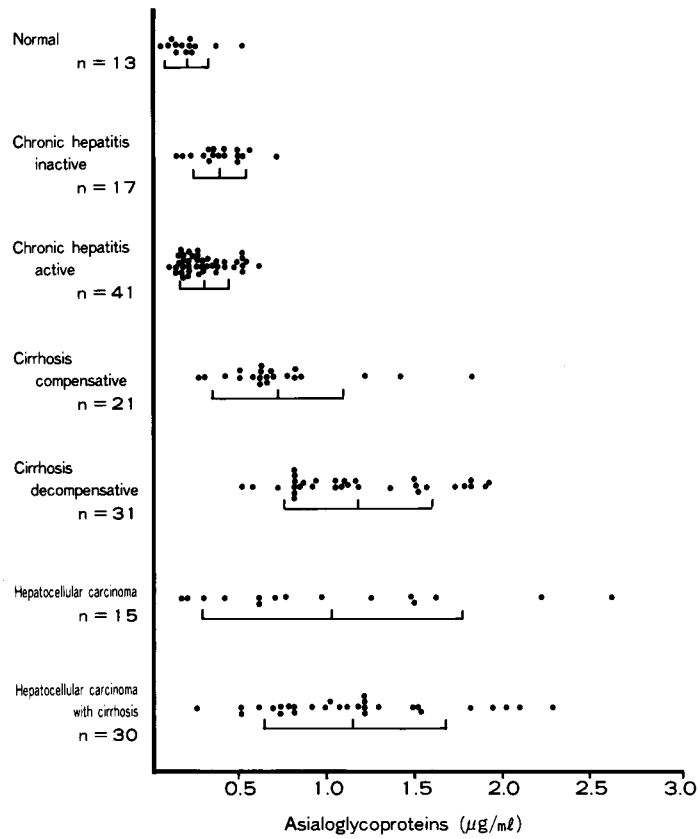

Fig. 2. The amount of asialoglycoproteins in serum of patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma.

Serum samples were dialyzed against 0.01 M Tris-HCl buffer, pH 7.8. The amount of asialoglycoproteins was determined by the competitive radioreceptor assay as described in Materials and Methods, and expressed in terms of micrograms of asialoorosomucoid required to cause the equivalent inhibition of the receptor activity.

Fig. 1, there was no interference of bilirubin or bile acids with the receptor activity.

Other Assays

The conventional liver function tests carried out in the hospital laboratory were as follows: Glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactose dehydrogenase (LDH), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), gamma glutamyltranspeptidase (γ-GTP), cholinesterase (ChE), total bilirubin, total cholesterol, the ratio of esterified cholesterol to total cholesterol (E/T cholesterol), prothrombin time, α-fetoprotein, ammonia, serum protein and its fraction, and indocyanine green retention test at 15 min (ICG-R_{15}). The latter was carried out as follows: The patients were intravenously administered indocyanine green 0.5 mg per kg body weight and serum retention at 15 min after injection of the dye was determined spectrophotometrically. In some patients given the 100 g oral glucose tolerance test within one week after sampling of the serum, glucose, immunoreactive insulin (IRI), and immunoreactive glucagon (IRG) were measured. The insulinogenic index was determined according to the formula described by Seltzer et al.[16] Summation of IRI and IRG was also performed.

Statistical analysis was determined by Student's t test and linear regression analysis and the results expressed as means ± S.D.

Results

Amounts of Serum Asialoglycoproteins

Figure 2 shows the amount of asialoglycoproteins in the serum of patients with chronic liver disease, with or without hepatocellular carcinoma and in control subjects. The mean level of asialoglycoproteins in the control serum was 0.191 ± 0.122 μg equivalent of asialoorosomucoid per ml and that in sera of patients with various liver diseases was as follows: Chronic inactive hepatitis; 0.373 ± 0.148, chronic active hepatitis; 0.248 ± 0.136, compensated cirrhosis; 0.707 ± 0.368, decompensated cirrhosis; 1.154 ± 0.414, hepatocellular carcinoma; 1.014 ± 0.736, hepatocellular carcinoma with cirrhosis; 1.142 ± 0.514. In patients with chronic hepatitis, the average level of asialoglycoproteins was elevated slightly and was higher than that in the controls ($p<0.01$). There was no significant difference between findings in cases of active and inactive chronic hepatitis. Marked accumulation of asialoglycoproteins was observed in patients with cirrhosis and hepatocellular carcinoma, as compared with control subjects ($p<0.001$). Serum asialoglycoprotein level in patients with decompensated cirrhosis was higher than that in patients with compensated cirrhosis ($p<0.001$).

Correlation between the Amount of Asialoglycoproteins and Liver Function Tests

The amount of serum asialoglycoproteins was determined in patients with chronic hepatitis, those with cirrhosis and in cases of hepatocellular carcinoma. The findings paralleled the data obtained from conventional liver function tests in which fresh serum was used.

Figure 3 shows that asialoglycoprotein levels correlated well with the value of albumin, ChE, ICG-R_{15} and the ratio of esterified cholesterol to total cholesterol ($p<0.001$). This figure also shows that the values of asialoglycoproteins and each test in chronic hepatitis were distributed in nearly normal ranges for both tests, while those in cirrhosis and hepatocellular carcinoma were scattered widely along the linear regression curve. The correlation coefficients between the amount of asialoglycoproteins and the levels of serum protein ($n=150$, $r=-0.480$), prothrombin time ($n=129$, $r=0.410$), fasting IRG ($n=54$, $r=0.561$), gamma globulin ($n=147$, $r=0.337$), total cholesterol ($n=145$, $r=-0.314$), ammonia ($n=65$, $r=0.429$) and ALP ($n=152$, $r=0.226$) were lower than those

shown in **Fig. 3** ($p<0.001$). There was a slight correlation between asialoglycoprotein level and the value of fasting IRI, summation of IRI and IRG, LDH and total bilirubin ($p<0.01$) (data not shown). No simple correlation existed among the levels of GOT, GPT, LAP, γ-GTP, α-fetoprotein, fasting glucose and the insulinogenic index.

A typical case of advancement from chronic hepatitis to cirrhosis in shown in **Fig. 4**. Hepatitis B surface antigen and antibody were not detected. The etiology in this patient was not determined. Jaundice was observed in September and December 1979, and ascitis occurred in June, 1980. Serum asialoglycoprotein level was 0.31 μg/ml in September, 1978 and increased to 1.5 μg/ml in September, 1980 with advanced clinical manifestations. Asialo-

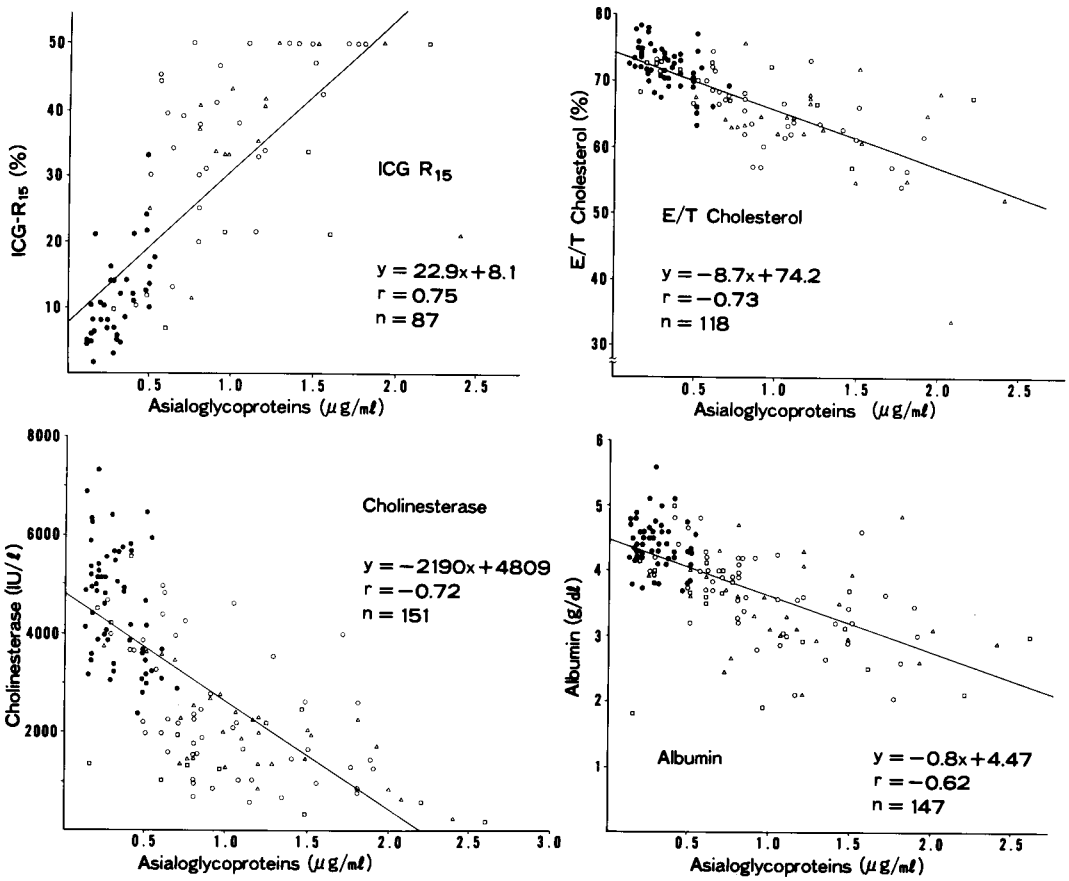

Fig. 3. Correlation between the amount of asialoglycoproteins and albumin content, cholinesterase activity, the ratio of esterified cholesterol to total cholesterol and indocyanine green retention test at 15 min.

Serum samples obtained from patients with chronic liver diseases with or without hepatocellular carcinoma were divided to two aliquots and each was used for the determination of asialoglycoproteins and for liver function tests, in the hospital laboratory. The relationships were examined by linear regression analysis.

● chronic hepatitis, ○ cirrhosis, □ hepatocellular carcinoma, △ hepatocellular carcinoma with cirrhosis

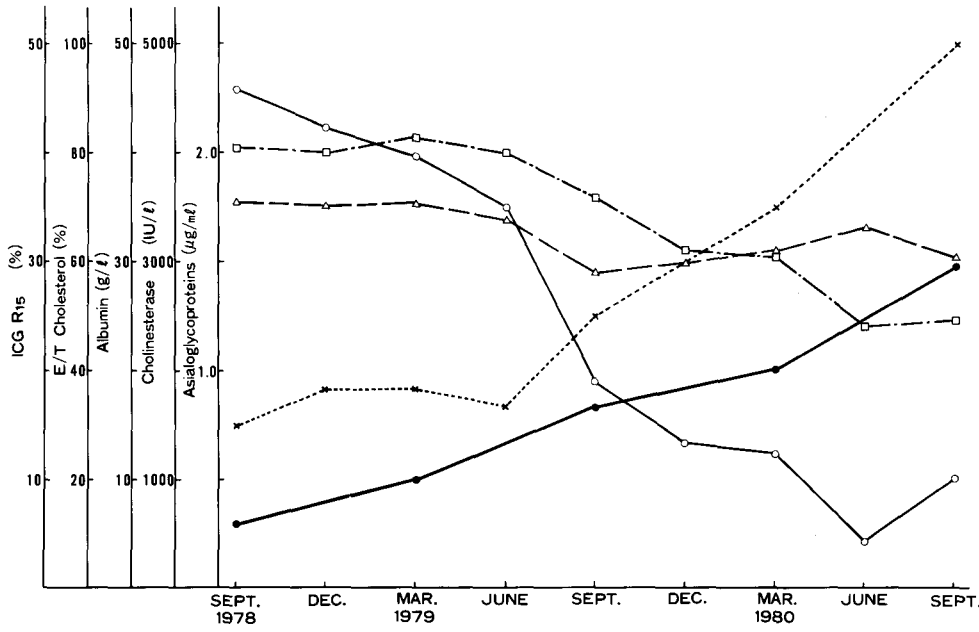

Fig. 4. The temporal changes in asialoglycoprotein levels and the results of conventional liver function tests in a patient with chronic hepatitis that advanced to cirrhosis.
●—● asialoglycoproteins, ×---× indocyanine green retention test at 15 min, □-·-□ albumin, △--△ the ratio of esterified cholesterol to total cholesterol, ○—○ cholinesterase

glycoproteins also accumulated in parallel with progression in the decrease of albumin content, ChE activity, the ratio of esterified cholesterol to total cholesterol, and the increase of ICG-R_{15}.

Correlation with Survival Time

The relationship between the amount of asialoglycoproteins and survival time was investigated in 29 patients with cirrhosis and/or hepatocellular carcinoma. A strong correlation was observed among these factors ($p<0.001$) (**Fig. 5**). There was a slight correlation between survival time and the values of ICG-R_{15} ($n=25$, $r=-0.381$), prothrombin time ($n=35$, $r=-0.339$) and the ratio of esterified cholesterol to total cholesterol ($n=29$, $r=0.385$) ($p<0.05$). No simple correlation exsisted between levels of ChE ($n=43$, $r=0.064$) and albumin ($n=42$, $r=0.276$). Most patients with an asialoglycoprotein level over 1 μg/ml died within 18 months.

As shown in **Figs. 2, 3** and **4**, the amount of asialoglycoproteins appears to be elevated in accordance with the progress of chronic liver diseases. The amount of asialoglycoproteins did not change in patients with chronic hepatitis with neither advancement nor improvement of the disease. In those dying of liver failure, asialoglycoprotein levels in cases of cirrhosis and/or hepatocellular carcinoma increased 1.5-3.5 times over levels determined on the first admission.

Discussion

Serum asialoglycoproteins are recognized by the receptors and taken up into hepatocytes to be catabolized in the lysosomes[1,2]. The present work showed that finite levels of asialoglycoproteins were detectable in normal serum, that the levels are greatly elevated in patients with cir-

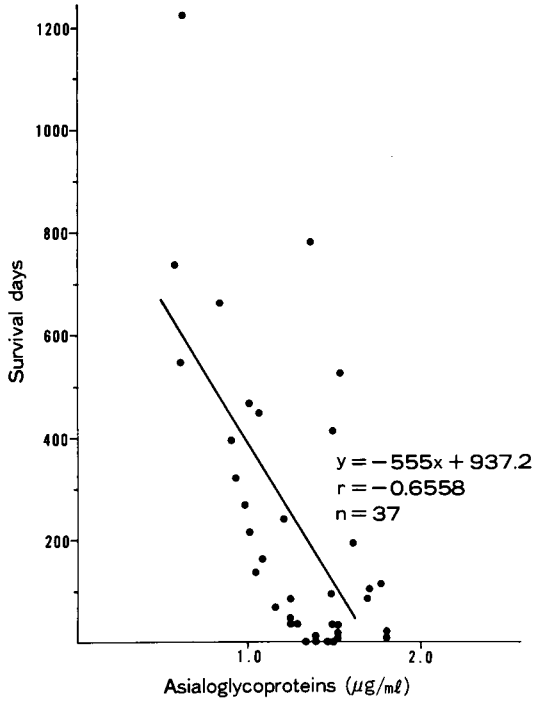

Fig. 5. Relationship between the amount of serum asialoglycoproteins and survival time of patients who died of cirrhosis or hepatocellular carcinoma.

rhosis and/or hepatocellular carcinoma, and that they were moderately elevated in those with chronic hepatitis (**Fig. 2**). These results are agreement with those reported by others[3-8].

Several investigators attempted to evaluate the clinical significance of measuring the amount of serum asialoglycoproteins in determining the diagnosis and prognosis of hepatic diseases[4-7]. We found that serum asialoglycoprotein levels correlated with the value of albumin, ChE, the ratio of esterified cholesterol to total cholesterol and ICG-R_{15} (**Fig. 3**), and were moderately correlated with the level of prothrombin time, fasting IRG, total cholesterol and gamma globulin. There was no relationship with GOT, GPT and α-fetoprotein. These data agree with reports that the amount of asialoglycoproteins correlated with the clearance rate of indocyanine green, albumin content[6] and ^{14}C-aminopyrine breath test[4], but not with GOT and GPT[4,6] in patients with cirrhosis. Thus, the amount of asialoglycoproteins appears to be in inverse proportion to hepatic functional reserve and to reflect the progress of chronic liver diseases. This conclusion seems to be supported by our previous observation that serum asialoglycoprotein levels were in inverse proportion to the amount of asialoglycoprotein receptors and that the receptor level depended on the hepatic functional mass[10]. In a typical case of advancement from chronic hepatitis to cirrhosis, the asialoglycoprotein level was elevated in accordance with the progress of the disease and correlated with the decrease in albumin content, ChE activity, the ratio of esterified cholesterol to total cholesterol and to the increase of ICG-R_{15} (**Fig. 4**).

Although several tests are useful to estimate residual liver function, it is difficult to interpretation their results and clinical performance. For example, the clearance rate of indocyanine green is one of the most common and reliable tests to estimate the functional mass of the liver[17-19]. The hepatic uptake of this dye, however, is interfered with by bilirubin when the concentration of bilirubin is over 3 mg/dl[20]. Neither interference by bilirubin nor bile acids was observed in the asialoglycoproteins assay (**Fig. 1**). Albumin content and ChE activity are easily measured but do vary when human albumin or fresh frozen human plasma is administered to patients intravenously. Asialoglycoprotein level is not affected by those medications, since the level is very low in normal plasma and the desialylation of serum glycoproteins is independent of the amount in serum[8], even if the amount of serum glycoproteins increases with administration of fresh frozen human plasma.

We conclude that the measurement of serum asialoglycoproteins can be used as a liver func-

tion test for estimating the functional reserve of the liver in patients with chronic liver diseases, with or without hepatocellular carcinoma.

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