Hepatic clearance measured with (99m)Tc-GSA single-photon emission computed tomography to estimate liver fibrosis.

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Received: February 12, 2014 Revised: June 3, 2014 Accepted: July 22, 2014 Published online: November 28, 2014

Abstract

AIM: To evaluate the clinical utility of hepatic clearance (HC) measured with technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin ($^{99m}$Tc-GSA) single-photon emission computed tomography (SPECT) to estimate the degree of liver fibrosis.

METHODS: Seventy-eight consecutive patients who underwent initial hepatectomy due to hepatocellular carcinoma were enrolled in this study. Indocyanine green clearance (ICG R15), quantitative indices estimated by $^{99m}$Tc-GSA [the receptor index (LHL15 and HH15) and HC via SPECT analysis], and conventional liver function tests were performed before hepatectomy. Correlations among the quantitative indices for liver functional reserve, conventional liver function tests, and the degree of liver fibrosis were evaluated.

RESULTS: The degree of liver fibrosis was correlated with ICG R15, HH15, LHL15, and HC. HC showed the best correlation with conventional liver function tests. According to multivariate analysis, HC and LHL15 were significant independent predictors of severe fibrosis. HC was the most valuable index for predicting severe fibrosis.

CONCLUSION: HC measured with $^{99m}$Tc-GSA SPECT is a reliable index for assessing liver fibrosis before hepatectomy.

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Key words: Fibrosis; Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin; Single-photon emission computed tomography; Hepatic clearance; Liver resection

Core tip: This retrospective study evaluated the clinical utility of hepatic clearance measured with technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin ($^{99m}$Tc-GSA) single-photon emission computed tomography for estimating the degree of liver fibrosis. We demonstrated that $^{99m}$Tc-GSA hepatic clearance showed strong correlations with the degree of liver fibrosis and conventional liver function tests. It is a reliable index for assessing severe liver fibrosis. We believe that this quantitative index can yield a more accurate estimation of liver fibrosis compared with currently used measures before hepatectomy for hepatobiliary surgeons.
INTRODUCTION
Liver fibrosis is a negative predictive factor for postoperative hepatic failure\[4,9\]. Cirrhosis is a well-known risk factor for postoperative hepatic failure\[13,4\]. Moreover, morbidity and mortality are high for patients with severe liver fibrosis undergoing liver resection\[6,8\]. Therefore, the accurate preoperative estimation of the extent of hepatic fibrosis is essential for successful liver surgery. Although many liver fibrosis indicators have been proposed for preoperative evaluation\[7-12\], the best indicator for evaluating liver fibrosis has not yet been established

Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin (99mTc-GSA) liver scintigraphy reflects the liver functional reserve and is reported to correlate with several hepatic function tests\[11,12\]. However, few available analyses can determine the degree of liver fibrosis. Single-photon emission computed tomography (SPECT) analysis in 99mTc-GSA liver scintigraphy, which can evaluate GSA accumulation in the liver, was also developed to investigate liver function\[10\]. These analyses calculate hepatic clearance (HC) with the outline extraction method, using a program based on a radio-pharmacokinetic model, as described by Shuke et al\[14,15\].

In this study, we investigate the contribution of HC measured with 99mTc-GSA SPECT to assess liver fibrosis.

MATERIALS AND METHODS

Patients
Between January 2011 and March 2014, 78 consecutive patients who underwent an initial hepatectomy due to hepatocellular carcinoma were enrolled in this study. The surgery was performed within 1 wk after 99mTc-GSA liver scintigraphy examination, and conventional tests were performed. All procedures were performed after informed consent was received from the patients and after approval from the Ethics Committee of Asahikawa Medical University Hospital was obtained. This study was performed in accordance with the ethical standards established in the 1964 Declaration of Helsinki.

99mTc-GSA liver scintigraphy and the receptor index
99mTc-GSA liver scintigraphy was scheduled for the patients on the day before their heptectomy. 99mTc-GSA was supplied by Nihon Medi-Physics (Nishinomiya, Japan). After the intravenous injection of 185 MBq 99mTc-GSA, dynamic imaging was performed with the patient in the supine position. LH15 was calculated by dividing the radioactivity of the region of interest (ROI) of the liver by the radioactivity of the ROI of the liver and the heart 15 min after injection. HH15 was calculated by dividing the radioactivity of the ROI of the heart 15 min after injection by the radioactivity of the ROI of the heart 3 min after injection\[16,17\].

SPECT analysis in 99mTc-GSA liver scintigraphy
Dynamic SPECT was performed using a dual-head gamma camera system equipped with low-energy, general-purpose collimators and a dedicated data processing unit (Millennium VG, GE, Tokyo, Japan). The in-plane spatial resolution of this system was 14 mm full width at half-maximum. After fasting overnight, the patient was placed in a supine position to ensure that the liver and lower part of the heart were within the detectors’ field of view. 99mTc-GSA (185 MBq) was injected intravenously as a bolus. After it was confirmed that the entire liver was covered by the detector’s view, dynamic SPECT data acquisition was started 1 min after injection and continued for 20 rotations in a 180° continuous rotation mode with an acquisition time of 1 min per rotation. In each rotation, the data from 60 projections were recorded in a 64 × 64 matrix (pixel size = 68.84 mm × 8.84 mm). SPECT images were reconstructed with a filtered back-projection method using a ramp filter after preprocessing with a Butterworth filter (cutoff frequency = 0.40 cycle per centimeter; order of 8) to obtain 8.84-mm-thick transaxial SPECT images. HC was determined from the SPECT data and was calculated with the outline extraction method using a program based on a radio-pharmacokinetic model, as described by Shuke et al\[14,15\].

Conventional liver function tests
The serum albumin (Alb), total bilirubin (T-bil), and cholinesterase (Ch-E) levels; prothrombin time international normalized ratio (PT-INR); and platelet count (Plt) were measured in the peripheral blood before heptectomy. The indocyanine green (ICG) test was conducted preoperatively, and the ICG clearance (ICG R15) was calculated using standard methods. The model for end-stage liver disease (MELD) score\[19\] and the Child-Turcotte-Pugh (CTP) score\[19\] were used as indices of liver dysfunctions.

Histopathological features of liver specimens
Liver fibrosis was diagnosed using surgical specimens, which were resected at a distance from the tumors. The degree of hepatic fibrosis was assessed and graded 0-6 according to the Ishak classification for chronic hepatitis\[8]: 0: no fibrosis; 1: fibrous expansion of some portal areas, with or without short fibrous septa; 2: fibrous expansion of most portal areas, with or without short fibrous septa; 3: fibrous expansion of most portal areas with occasional portal-to-portal bridging; 4: fibrous expansion of portal areas with marked bridging (portal to portal as well as portal to central); 5: marked bridging (portal to portal and/or portal to central) with occasional nodules; and 6, cirrhosis, probable or definite. Scores of 0, 1, 2, and 3 were considered to reflect nonsevere fibrosis. Scores of 4, 5, and 6 were recorded as severe fibrosis. Tumor size, tumor number, and tumor vascular invasion (portal vein, hepatic artery, and hepatic vein) were evaluated using surgical specimens.
The degree of liver fibrosis was correlated with ICG R15, HH15, and HC. ICG R15: Indocyanine green dye retention at 15 min; HC: Hepatic clearance.

**Correlations between quantitative indices for liver functional reserve and conventional liver function tests**

As Table 3 shows, we evaluated the correlations between the preoperative parameters for liver function and conventional liver function tests. LHL15 was correlated with platelet count ($r = 0.235$, $P = 0.038$) and albumin level ($r = 0.263$, $P = 0.020$), and HH15 was correlated with total bilirubin level ($r = 0.289$, $P = 0.010$) and cholinesterase level ($r = -0.263$, $P = 0.020$). HC was correlated with all conventional liver function tests after liver resection: platelet count ($r = 0.348$, $P = 0.002$), prothrombin time ($r = -0.287$, $P = 0.011$), albumin level ($r = 0.233$, $P = 0.040$), total bilirubin level ($r = -0.345$, $P = 0.002$), and cholinesterase level ($r = -0.419$, $P = 0.0001$).

**Univariate and multivariate stepwise regression analysis of various factors affecting liver fibrosis**

Univariate analysis showed that platelet count ($P < 0.001$), prothrombin time ($P = 0.032$), total bilirubin level ($P = 0.001$), tumor size ($P = 0.042$), MELD score ($P = 0.009$), ICG R15 ($P = 0.019$), LHL15 ($P = 0.042$), HH15 ($P = 0.0004$), and HC ($P < 0.0001$) were significant predictors of severe cirrhosis. When we entered platelet count, prothrombin time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC into a multivariate logistic regression model to identify variables with independent predictive value for severe fibrosis, we found that HC and LHL15 were the significant independent predictors (Table 4).

**ROC curve and interactive dot diagrams of HC and LHL15 for the diagnosis of severe fibrosis**

In Figure 1, we present the ROC curves for each of the 2 variables, HC and LHL15, that were identified as the significant independent predictors of severe fibrosis. The AUC of the ROC curves for HC and LHL15 were 0.826 and 0.641, respectively. There was a significant difference between the two values ($P = 0.0146$). Based on the analysis employing interactive dot diagrams, the cutoff values for predicting severe cirrhosis with the highest sensitivity and specificity were 298 (sensitivity, 77.8%; specificity, 84.3%) for HC and 0.926 (sensitivity, 74.1%; specificity, 60.8%) for LHL15.
Table 3  Correlations between quantitative indices for liver functional reserve and conventional liver function tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>ICG R15</th>
<th>LHL 15</th>
<th>HH 15</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P value</td>
<td>r</td>
<td>P value</td>
</tr>
<tr>
<td>Platelets (x 10^9/mm³)</td>
<td>-0.160</td>
<td>0.161</td>
<td>0.235</td>
<td>0.038</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>0.082</td>
<td>0.473</td>
<td>-0.122</td>
<td>0.289</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>-0.044</td>
<td>0.703</td>
<td>0.263</td>
<td>0.020</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.204</td>
<td>0.073</td>
<td>-0.217</td>
<td>0.057</td>
</tr>
<tr>
<td>Cholinesterase (U/L)</td>
<td>-0.113</td>
<td>0.324</td>
<td>0.221</td>
<td>0.052</td>
</tr>
</tbody>
</table>

LHL15 was correlated with platelet count and albumin level. HH15 was correlated with total bilirubin level and cholinesterase level. HC was correlated with all conventional liver function tests. ICG R15: Indocyanine green dye retention at 15 min; HC: Hepatic clearance.

Table 4  Univariate and multivariate analyses of variables predictive of severe fibrosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe fibrosis (n = 27)</th>
<th>No (n = 51)</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>25/4</td>
<td>Nov-40</td>
<td>0.474</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>66.5 ± 10.0</td>
<td>66.8 ± 10.5</td>
<td>0.950</td>
<td></td>
</tr>
<tr>
<td>HBs-Ag (+/-)</td>
<td>10/17</td>
<td>16/35</td>
<td>0.616</td>
<td></td>
</tr>
<tr>
<td>HCV-Ab (+/-)</td>
<td>10/17</td>
<td>11/40</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>Alcohol abuse (+/-)</td>
<td>2/25</td>
<td>8/43</td>
<td>0.301</td>
<td></td>
</tr>
<tr>
<td>NASH (+/-)</td>
<td>4/23</td>
<td>10/41</td>
<td>0.602</td>
<td></td>
</tr>
<tr>
<td>Platelets (x 10^9/mm³)</td>
<td>12.7 ± 3.9</td>
<td>18.7 ± 7.4</td>
<td>&lt;0.001</td>
<td>0.096</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>1.09 ± 0.12</td>
<td>1.03 ± 0.10</td>
<td>0.032</td>
<td>0.223</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0 ± 0.6</td>
<td>4.1 ± 0.6</td>
<td>0.388</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>0.001</td>
<td>0.354</td>
</tr>
<tr>
<td>Cholinesterase (U/L)</td>
<td>234 ± 75</td>
<td>255 ± 68</td>
<td>0.229</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>3.5 ± 1.6</td>
<td>5.7 ± 4.2</td>
<td>0.042</td>
<td>0.137</td>
</tr>
<tr>
<td>Tumor number</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.6</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td>Tumor vascular invasion (+/-)</td>
<td>5/22</td>
<td>16/35</td>
<td>0.226</td>
<td></td>
</tr>
<tr>
<td>MELD score</td>
<td>5.8 ± 1.1</td>
<td>5.1 ± 1.2</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>CTP score</td>
<td>5.3 ± 0.6</td>
<td>5.2 ± 0.4</td>
<td>0.685</td>
<td></td>
</tr>
<tr>
<td>ICG R15 (%)</td>
<td>14.3 ± 6.1</td>
<td>10.2 ± 5.5</td>
<td>0.019</td>
<td>0.183</td>
</tr>
<tr>
<td>LHL 15</td>
<td>0.901 ± 0.044</td>
<td>0.935 ± 0.024</td>
<td>0.042</td>
<td>0.041</td>
</tr>
<tr>
<td>HH 15</td>
<td>0.648 ± 0.068</td>
<td>0.556 ± 0.067</td>
<td>0.004</td>
<td>0.053</td>
</tr>
<tr>
<td>HC</td>
<td>263.3 ± 90.4</td>
<td>381.1 ± 96.7</td>
<td>&lt;0.001</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Platelet count, prothrombin time, total bilirubin level, tumor size, MELD score, ICG R15, LHL15, HH15, and HC were significant predictors of severe cirrhosis in the univariate analysis. In the multivariate analysis, HC and LHL15 were the significant independent predictors. HBs-Ag: Hepatitis B surface antigen; HCV-Ab: Hepatitis C virus antibody; NASH: Nonalcoholic steatohepatitis; MELD score: Model for end-stage liver disease score; CTP score: Child-Turcotte-Pugh score; ICG R15: Indocyanine green dye retention at 15 min; HC: Hepatic clearance.

**DISCUSSION**

In the current study, we demonstrated correlations between the degree of liver fibrosis and ICG R15, HH15, LHL15, and HC. Among these indicators, HC showed the best correlation with conventional liver function tests. HC was the most valuable index for predicting severe cirrhosis. An HC of 298 could be used to predict severe cirrhosis.

The degree of liver fibrosis is a negative predictor of liver regeneration and the restoration of liver function after liver resection\(^9\). Therefore, estimating the liver functional reserve, which is a reflection of liver fibrosis, is important. Several laboratory variables, such as prothrombin time and cholinesterase, have prognostic value in chronic liver disease\(^21\). In addition, the Alb level, T-bil level, and prothrombin time are the most useful routine laboratory tests for establishing a prognosis for hepatitis patients\(^25\). However, none of these laboratory variables reflects liver fibrosis directly. As a result, these variables cannot be used as indices for determining the extent of liver resection for patients with liver tumors. In contrast, several studies have evaluated the liver functional reserve before hepatectomy\(^23-25\). In particular, the indocyanine green (ICG) clearance test has been widely used to evaluate liver functional reserve\(^25,26\) for liver resection. However, it does not provide quantitative parameters. Moreover, there are occasional discrepancies between the ICG clearance values and histologic findings in the liver because of the imbalance of portal inflow or portasystemic shunts. Such discrepancies make direct assessments of the extent of liver fibrosis difficult. Therefore, a new method to estimate the liver functional reserve that accurately reflects the degree of fibrosis is required.

The asialoglycoprotein receptor (ASGPR) is localized on hepatocytes and is involved in the clearance of glycoproteins containing terminal galactose residues from the circulation\(^27,28\). The expression of this receptor decreases according to the number of functional hepatocytes. Therefore, liver scintigraphy with \(^99\)Tc-GSA, ...
an analog of asialoglycoprotein, enables the quantitative evaluation of liver functional reserve. SPECT analysis in 99mTc-GSA liver scintigraphy, which allows the evaluation of GSA accumulation in the liver, was also developed to investigate liver function\(^{[19]}\). 99mTc-GSA HC, which is determined based on SPECT data, demonstrates the precise distribution of ASGPR in the liver, thereby providing an accurate calculation of liver functional reserve\(^{[20]}\). In this study, 99mTc-GSA HC showed a correlation with conventional liver function tests and the extent of liver fibrosis that was better than that of LHL15 or HH15. LHL15 and HH15, which are hepatic uptake and blood clearance ratios in 99mTc-GSA liver scintigraphy, are the simplest and most commonly used variables. However, they may be insufficient for accurately estimating the degree of liver fibrosis because these indices are calculated from planar scintigraphic images, which do not correctly reflect hepatocyte volume. In contrast, 99mTc-GSA HC measured by SPECT analysis contains volumetric information and may correctly estimate the hepatocyte volume, thus reflecting the degree of liver fibrosis.

In liver surgery, the risk of perioperative complications is generally believed to increase when the remnant liver volume (RLV) is excessively small\(^{[30]}\). Therefore, reports have advocated preoperatively assessing RLV with CT volumetry\(^{[31]}\). However, CT volumetry can never reflect the function of the remnant liver, especially in patients with parenchymal disease\(^{[30,32]}\), such as chronic hepatitis or cirrhosis. Additionally, several reports concerning 99mTc-GSA SPECT findings have indicated that regional function is not necessarily uniform throughout the liver\(^{[33,34]}\), suggesting that an accurate estimation of regional liver function is more important for predicting postoperative liver functional reserve. In this study, 99mTc-GSA HC strongly reflected the degree of liver fibrosis. Therefore, we believe that using the combined 99mTc-GSA HC and CT volumetric measurements of the remnant liver can evaluate remnant liver functional reserve after hepatectomy\(^{[35]}\). Further studies are needed to test this hypothesis.

In conclusion, we demonstrated that HC measured with 99mTc-GSA SPECT showed correlations with the degree of liver fibrosis and conventional liver function tests. 99mTc-GSA HC was the most valuable index for predicting severe fibrosis. It could yield a more accurate estimation of liver fibrosis compared with currently used measures before hepatectomy for hepatobiliary surgeons.

**COMMENTS**

**Background**

Liver fibrosis is a negative predictive factor for postoperative hepatic failure. Therefore, the accurate preoperative estimation of the extent of hepatic fibrosis is essential for successful liver surgery. Although many liver fibrosis indicators have been proposed for preoperative evaluation, the best indicator for evaluating liver fibrosis has not yet been established.

**Research frontiers**

Technetium-99m-diethylenetriaminepenta-acetic acid-galactosyl human serum albumin (99mTc-GSA) liver scintigraphy reflects the liver functional reserve and is reported to correlate with several hepatic function tests. In addition, single-photon emission computed tomography analysis in 99mTc-GSA liver scintigraphy, which can evaluate GSA accumulation in the liver, was also developed to investigate liver function.

**Innovations and breakthroughs**

Hepatic clearance which was measured with 99mTc-GSA single-photon emission computed tomography (SPECT) is a reliable index for assessing liver fibrosis.

**Applications**

Hepatic clearance which was measured with 99mTc-GSA SPECT could yield a
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P- Reviewer: El-Sayed M, Welling TH S- Editor: Ma YJ
L- Editor: A E- Editor: Wang CH