Biomarkers of Liver Hypertrophy after Balloon-occluded Retrograde Transvenous Obliteration

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Abstract

PURPOSE: This study explored the potential biomarkers correlated with liver hypertrophy after balloon-occluded retrograde transvenous obliteration (BRTO).

MATERIALS AND METHODS: The liver volume was calculated using volumetry with contrast-enhanced computed tomography (CT) images before and 1 month after BRTO in 18 patients (10 men, 8 women) with a median age of 66.9 years (range, 38-78 years). Patients whose liver volume increased by more than 5% after BRTO were categorized as group A, and the other patients were categorized as group B. Correlation between variables such as patient background, liver function test, and percentage increase of the alpha-fetoprotein (AFP) level and liver volume were evaluated using Pearson’s correlation coefficient. The performance of a potential biomarker of liver hypertrophy was evaluated by the receiver operating characteristic (ROC) analysis. Moreover, changes in liver profiles following BRTO were assessed in each patient group.

RESULTS: The liver volume increased by more than 5% in 7 patients (39%, 7/18; group A) after BRTO. Only the percentage increase of AFP level (r=0.77, p<0.01) was significantly correlated with the percentage increase of liver volume. The area under the ROC curve (AUC) value for percentage increase of AFP level was 0.86 (95% confident interval, 0.65-1.00) with an optimal cutoff value of 4.7%. Significant improvement in both prothrombin time (p<0.05) and Child-Pugh score (p<0.04) was observed following BRTO in group A. However, the liver profiles remained unchanged in group B.

CONCLUSION: The percentage increase of AFP level can be used as a biomarker of liver hypertrophy after BRTO, leading to liver profile improvement.

Key words: BRTO, Liver Hypertrophy, AFP, Liver profiles

INTRODUCTION

Balloon-occluded retrograde transvenous obliteration (BRTO) is a useful therapeutic option for the treatment of gastric varices and hepatic encephalopathy [1]. Recent reports have described that BRTO can cause liver hypertrophy by increasing portal blood flow [2]. However, no liver hypertrophy was observed among all patients after BRTO. The characteristics of patients with liver hypertrophy remain unclear [2]. Moreover, no relevant literature review described a study examining the relationship between liver hypertrophy and liver profiles.

Alpha-fetoprotein (AFP) is reportedly a marker reflecting liver regeneration after a liver injury [3-5]. Therefore, blood tests such as AFP can be used as biomarkers to predict liver hypertrophy following BRTO. Finding such clinical biomarkers is important for choosing the optimal treatment strategy and estimating the patient prognosis after BRTO.

Therefore, this study explored the potential biomarkers...
correlated with liver hypertrophy following BRTO. The relationship between changes in liver volume and liver profiles was also evaluated.

**MATERIALS AND METHODS**

**Patients**

Our institutional review board approved this retrospective study. Written informed consent to take part in this study was waived because of its retrospective nature.

Between January 2008 and October 2017, 127 consecutive patients underwent BRTO for the treatment of gastric varices. Among them, patients who received a scheduled liver functional test, AFP evaluation, and contrast-enhanced CT within 1 month before and after BRTO were included in the study. However, patients with hepatocellular carcinoma were excluded. A total of 18 patients were examined in this study. Approximately 109 patients (78.7%, 109/127) were excluded from this study due to the lack of contrast-enhanced CT imaging at 1 month after BRTO in 7 patients (6.4%, 7/109), lack of AFP evaluation in 39 patients (35.8%, 39/109), lack of both CT and AFP evaluation in 27 patients (24.8%, 27/109), and presence of hepatocellular carcinoma in 36 patients (33.0%, 36/109).

The subjects consisted of 10 men (55.6%, 10/18) and 8 women (44.4%, 8/18) with a median age of 66.9 years (range, 39-79 years). All patients had liver cirrhosis caused by hepatitis B or C viral infection (n=10), alcohol abuse (n=2), non-alcoholic steatohepatitis (n=1), primary biliary cholangitis (n=1), autoimmune hepatitis (n=1), and unknown (n=3). Eight patients (44.4%, 8/18) had a Child-Pugh class A liver profile. Ten patients (55.6%, 10/18) had class B liver profiles.

**BRTO**

BRTO was performed percutaneously under local anesthesia using lidocaine (Xylocaine®; AstraZeneca K.K., Osaka, Japan). After an 8 Fr guiding catheter (BRTO-ASA®; Medikit Co. Ltd., Tokyo, Japan) was introduced into the left renal vein via the right femoral vein, a 6 Fr balloon catheter (Selecon MP® Catheter 2; Terumo Clinical Supply Co. Ltd., Gifu, Japan) was inserted into the gastro-renal shunt. Then, a 1:1 mixture of 10% ethanolamine oleate (Oldamin®; Fuji Chemical Ind. Co. Ltd., Toyama, Japan) and contrast medium (Iopamiron®; Bayer Yakuhin, Ltd., Osaka, Japan) was injected into the gastric varices under balloon occlusion. The catheter system was removed on the next day after confirmation of complete occlusion of gastric varices by injecting a contrast medium via the balloon catheter.

**CT liver volumetry**

CT liver volumetry was performed using a multidetector-row CT (SOMATOM® Definition AS+; Siemens Healthcare Diagnostics, Erlangen, Germany) and analyzed using an application software (ZIOSTATION2®; Ziosoft Inc., Tokyo, Japan) (Figure 1). A contrast medium (Iopamiron®; Bayer Yakuhin, Ltd., Osaka, Japan) was injected via the antecubital vein with an injection rate of 3.0 mL/s. CT images were acquired using the following parameters: tube voltage of 100 kV, tube current of 100 mAs, collimation of 0.6 mm × 128, reconstruction thickness of 5 mm, gantry rotation time of 0.5 s, table feed speed of 46 mm/s, and pitch of 0.6. Image reconstruction was performed by the filtered black projec-
tion algorithm.

The liver areas on each CT section were calculated using manual tracing by 2 radiologists (JT and HT) with 2 and 19 years of experience, respectively. The total liver volume was calculated as the summed area of each CT section.

**Follow-up**

Physical and blood examination including complete blood count, blood biochemistry, and AFP level were performed within 1 month before and after BRTO among all patients. Furthermore, CT liver volumetry was performed within 1 month before or after BRTO. The therapeutic response of shunt occlusion and disappearance of gastric varices were evaluated using contrast-enhanced CT.

**Assessment and statistical analysis**

The patients were divided into 2 groups according to the liver volume change at 1 month after BRTO. Patients whose liver volume increased by more than 5% were categorized as group A. All other patients were categorized as group B. The characteristics of the 2 patient groups were compared using the Mann-Whitney U test for continuous variables or the Wilcoxon signed-rank test for categorical variables.

In this study, the patient’s age, baseline liver volume, and items that can be used to estimate the liver function such as the Child-Pugh score, albumin, total bilirubin, and prothrombin time were used as potential biomarkers of liver volume change after BRTO. In addition, baseline AFP and percentage increase of AFP level were used as potential biomarkers because AFP is reportedly a marker reflecting liver regeneration after a liver injury [3-5]. Correlations between those potential biomarkers and the percentage increase of liver volume after BRTO were evaluated using Pearson’s correlation coefficient. Benjamini-Hochberg correction for reduction in the false positive rate was used to evaluate the statistical significance [6]. If potential biomarker which correlates the liver hypertrophy after BRTO was found by Pearson’s correlation coefficient, the performance of a potential biomarker was evaluated by the receiver operating characteristic (ROC) analysis. The optimal cutoff value of potential biomarkers of liver volume increase was determined as the value of the maximum Youden index. Then, the sensitivity and specificity rates, positive predictive value (PPV), negative predictive value (NPV), and accuracy of optimal cutoff value were calculated. Improvements in the patients’ liver profiles were evaluated before and after BRTO in each patient group using a paired t-test.

All continuous data are expressed as median with ranges (minima-maxima). All p-values <0.05 were considered as statistically significant. All statistical analyses were conducted using a software (SAS, release 9.1; SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**BRTO**

BRTO was technically successful among all patients. Although major portal vein thrombosis and ascites were observed after BRTO in 1 patient (5.6%, 1/18), minor portal vein thrombosis was observed after BRTO in 4 patients (16.7%, 3/18). No other complications were observed. Complete occlusion of gastric varices was observed the next day after BRTO in all patients. Additionally, contrast-enhanced CT after BRTO showed thrombosis of gastric varices among all patients. Follow-up endoscopic examinations were performed in 12 patients, and remission of gastric varices was observed among all patients.

**Comparison of patient characteristics**

Among 18 patients who underwent BRTO, the liver volume increased by more than 5% in 7 patients at 1 month after BRTO (39%, 7/18; group A) (Figure 2). In the other 11 patients, no liver volume increase of more than 5% was noted (61%, 11/18; group B). No significant difference was found in the characteristics between the 2 patient groups (Table 1).

**Correlations between potential biomarkers and liver volume**

Pearson’s correlation coefficient showed that the percentage increase of AFP level (r=0.77, p<0.01) and pre-procedural bilirubin level (r=0.56, p<0.02) was correlated with the percentage increase of the liver volume (Table 2, Figure 3). Of these potential biomarkers, the latter was rejected by the control false discovery rate with Benjamini-Hochberg theory. Therefore, the percentage increase of AFP level was found to be the only significant factor correlated with the percentage increase of liver volume after BRTO (Table 2).
Table 1. Comparisons of baseline characteristics before BRTO between two groups

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
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<tbody>
<tr>
<td></td>
<td>n=7</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>68.9 (63-79)</td>
<td>65.7 (39-79)</td>
<td>0.96</td>
</tr>
<tr>
<td>≤65/ &gt; 65</td>
<td>3/4</td>
<td>3/8</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>3/4</td>
<td>7/4</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Child–Pugh score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>6.9 (5-9)</td>
<td>6.2 (5-9)</td>
<td>0.32</td>
</tr>
<tr>
<td>A/B</td>
<td>2/5</td>
<td>6/5</td>
<td>0.28</td>
</tr>
<tr>
<td><strong>Cause of cirrosis</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HCV/other</td>
<td>3/4</td>
<td>4/7</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Baseline liver volume (ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>993.4 (758.9–1237.1)</td>
<td>1156.5 (932.4–1885.8)</td>
<td>0.22</td>
</tr>
<tr>
<td>1000&lt;1000≥</td>
<td>4/3</td>
<td>8/3</td>
<td>0.21</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>3.5 (2.2–4.4)</td>
<td>3.8 (3.1–4.5)</td>
<td>0.36</td>
</tr>
<tr>
<td>3.5&lt;3.5&lt;</td>
<td>3/4</td>
<td>8/3</td>
<td>0.21</td>
</tr>
<tr>
<td>T-Bil (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.2 (0.7–2.0)</td>
<td>1.4 (0.5–3.9)</td>
<td>0.93</td>
</tr>
<tr>
<td>1&lt;1≥</td>
<td>4/3</td>
<td>6/5</td>
<td>0.91</td>
</tr>
<tr>
<td>PT (%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>68.7 (48.5–78.4)</td>
<td>75.4 (52.4–99.6)</td>
<td>0.56</td>
</tr>
<tr>
<td>70&lt;70≥</td>
<td>3/4</td>
<td>6/5</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>PT-INR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.23 (1.19-1.5)</td>
<td>1.2 (1-1.52)</td>
<td>0.72</td>
</tr>
<tr>
<td>≤1.2&gt;1.2</td>
<td>5/2</td>
<td>6/5</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Baseline AFP (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>5.4 (3.1–13.5)</td>
<td>6.5 (1.9–14.1)</td>
<td>0.68</td>
</tr>
<tr>
<td>5&lt;5≤</td>
<td>2/5</td>
<td>6/5</td>
<td>0.28</td>
</tr>
</tbody>
</table>

BRTO, balloon-occluded transvenous obliteration; HCV, hepatitis c virus; T-Bil, total bilirubin; PT, prothrombin time; PT-INR, prothrombin time-international normalized ratio; AFP, alpha-fetoprotein

**Performance of the percentage increase of AFP level for liver volume increase after BRTO**

The area under the ROC curve (AUC) value for the percentage increase of AFP level for correlating the percentage increase of the liver volume after BRTO was 0.86 [95% confidence interval (CI), 0.65-1.00] with an optimal cutoff value of 4.7%. When this cutoff value was applied, the sensitivity and specificity rates, PPV, NPV, and accuracy became 85.7% (95% CI, 42.1-99.6%), 72.7% (95% CI, 39.0-94.0%), 66.7% (95% CI, 29.9-92.5%), 88.9% (95% CI, 51.8-99.7%), and 77.8% (95% CI, 52.4-93.6%), respectively.

**Relation between liver volume change, liver function, and AFP**

In patients with liver hypertrophy (group A), the prothrombin time increased significantly (p<0.05), with significant improvement in the Child-Pugh score (p<0.04) at 1 month after BRTO (Table 3). The patients’ liver profiles remained unchanged at 1 month after BRTO in patients without liver hypertrophy (group B) (Table 3). The median AFP level increased from 5.4 ng/mL (range, 3.1-13.5 ng/mL) to 6.7 ng/mL (range, 3.5-15.4 ng/mL) after BRTO in group A. On the other hand, the median AFP level decreased from
Figure 3. Receiver operating characteristic curve of the percentage increase of AFP level for correlating liver volume increase after balloon-occluded retrograde transvenous obliteration. The dash line corresponds to the 1:1 correlation between the 2 parameters.

6.5 ng/mL (range, 1.9-14.1 ng/mL) to 5.0 ng/mL (range, 1.8-8.6 ng/mL) in group B.

Table 2. Correlation between liver volume change and biomarkers

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>P</th>
<th>FDR corrected P</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>0.05</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>Child–Pugh score</td>
<td>-0.02</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Baseline liver volume (ml)</td>
<td>-0.2</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>-0.28</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>T-Bil (mg/dl)</td>
<td>-0.56</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>PT (%)</td>
<td>-0.05</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>PT-INR</td>
<td>-0.1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Baseline AFP (ng/ml)</td>
<td>-0.42</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>% increase of AFP</td>
<td>0.77</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>

T-Bil, total bilirubin; PT, prothrombin time; PT-INR, prothrombin time-international normalised ratio; AFP, alpha-fetoprotein; FDR, false discovery rate *Statistically significant after correcting for multiple comparisons by Benjamini–Hochberg's correction.

DISCUSSION

Reportedly, BRTO can induce liver hypertrophy and improve liver function [2, 7]. Our study showed no liver hypertrophy among all patients who underwent BRTO. Although liver hypertrophy (increase of over 5%) was observed in 39% of patients who underwent BRTO, it was not found in 61% of patients 1 month after BRTO. Kako et al. reported that the average liver volume remained unchanged 1 month after portosystemic shunt occlusion [2].

Our study suggests that the percentage increase of AFP level may be used as a biomarker to estimate liver hypertrophy following BRTO. Serum AFP has been used primarily as a marker of hepatocellular carcinoma [3]. However, recent reports have described that higher AFP level is sometimes observed among patients with non-malignant liver disease such as hepatitis [3, 4]. Likewise, AFP is known as a marker of hepatic progenitor cells that are the source of hepatocytes during liver regeneration [5]. Therefore, a positive correlation between the percentage increase of AFP level and liver volume observed in this study might reflect liver regeneration following BRTO.

It is noteworthy that our results clearly demonstrated liver profile improvement among patients with liver hypertrophy. The function of individual hepatocytes is well-preserved even in patients with a cirrhotic liver [8, 9]. Therefore, in-
creased number of hepatocytes through liver regeneration might engender liver profile improvement after BRTO.

This study has several limitations. The small number of included patients, their inhomogeneous backgrounds, short follow-up periods, and manual liver volume measurement are readily apparent limitations. The use of the manual tracing method for the calculation of liver volume is another limitation. This method may be subjective and less accurate than the automated CT volume analyzing tools. Additionally, although AFP includes 3 glycoforms of AFP-L1, AFP-L2, and AFP-L3 according to their binding capacity to the lectin Lens culinaris agglutinin (LCA), this study only evaluated the total AFP levels [10]. AFP-L1 is the non-LCA-bound fraction which increases in patients with chronic hepatitis and liver cirrhosis without hepatocellular carcinoma [10]. Therefore, AFP-L1 might reflect liver volume increase more precisely than the total AFP level. Finally, the result of this study revealed that the optimal cutoff value for percentage increase of AFP level which is correlated with liver hypertrophy after BRTO was 4.7%. However, this cutoff value was not validated by subsequent analysis. Further study utilizing a larger patient population will be required.

In conclusion, the percentage increase of AFP level can be used as a biomarker of liver hypertrophy after BRTO, leading to liver profile improvement.

Conflict of interest: The authors declare that they have no conflicts of interest.

References


