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# Serum Level of Hyaluronic Acid Does not Correlate with Changes of Hepatic Volume after Portal Vein Embolization

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**The serum hyaluronic acid (HA) levels are associated with liver regeneration after hepatectomy. In the present study, the HA concentrations were examined to evaluate the relationship with changes of hepatic volume after right portal vein embolization (PVE). The HA level of serum samples from 10 patients who underwent PVE before hepatectomy of the right lobe was measured, and the results were compared to the changed volume of embolized right lobe and unembolized left lobe of the liver. The mean serum HA level in patients with chronic viral liver disease (CVLD) (202+/-118 ng/ml) was significantly greater than in those without CVLD (70+/-24 ng/ml) ($p<0.05$). The volume of embolized liver decreased 72+/-96 $cm^3$ (-8.9+/-5.5 %), while the volume of unembolized liver increased 106+/-67 $cm^3$ (+8.9+/-5.5 %) 2 weeks after PVE. HA concentrations after 2 weeks of PVE (296+/-216 ng/ml) tended to be greater than that before PVE (134+/-108 ng/ml) but not statistically significant ($p=0.105$). There were no correlations between serum HA levels before PVE and the changes of hepatic volume in embolized and unembolized lobe after PVE. Our results indicate that the measurement of HA level is not useful for predicting the effect of PVE.**

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**Key Words:** hyaluronic acid, portal vein embolization, regeneration

## Introduction

To overcome impaired hepatic functions after major hepatectomy, portal vein embolization (PVE) technique, which was firstly published by Makuuchi et al.,[1] has been applied to reduce the expected hepatic volume for resection and initiate hypertrophy of the future remnant liver. The preoperative PVE is safe and minimize postoperative liver failure after major surgery.[2,3] Hepatic functions of remnant liver are maintained after PVE.[4,5]

The serum level of hyaluronic acid (HA) is a sensitive marker of hepatic fibrosis and reflects the functional liver reserve in patients with chronic hepatitis or cirrhosis including our previous pilot study.[6−8] As markers of hepatic fibrosis, serum level of collagen or extra-cellular matrix, such as the type III aminoterminal peptide of procollagen (PIIIP) or laminin have been measured as well.[9,10] However, HA is specifically metabolized by endothelial cells of hepatic sinusoids and increased serum HA level reflects damage of hepatic endothelial cells.[11] Therefore, serum HA level reflects the functional liver reserve of non-parenchymal liver cells. Our study revealed that the serum HA level at pre- and post-operative state was significantly correlated with the regenerated volume of remnant liver after lobectomy.[8] This indicates that serum HA level is a useful marker for regeneration after major hepatectomy. Yachida et al. reported a correlation between serum HA level and hypertrophic ratio subsequent to PVE.[12]

In the present study, we also evaluated the usefulness of serum HA as an index of hypertrophy of remnant liver after PVE by measuring its level before right or extended right lobectomy of the liver.

## Patients and Methods

### *Patients*

The subjects were 10 patients with hepato-biliary diseases who underwent right lobe or extended right lobe hepatectomy in the First Department of Surgery, Nagasaki University School of Medicine between January 1996 and September 2001. They included 9

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males and a female with a mean age of 63 ± 7 years (range, 61-73 years). Hepato-biliary carcinoma included 5 hepatocellular carcinomas, 2 gall bladder carcinomas, 2 hilar bile duct cancers, and a intra-hepatic cholangiocarcinoma. The background liver abnormality included chronic viral liver diseases in 5, obstructive jaundice in 2 and liver with normal function in 3 patients. These patients were divided into two groups of chronic viral liver disease (CVLD) consisting of chronic hepatitis and cirrhosis (n=5), and non-CVLD consisting of normal and icteric liver (n=5).

In our institute, the volume of liver tissue to be resected during surgery is calculated based on the results of ICG R15 and Takasaki's formula.[13] The volume of the liver section to be resected excluding tumor volume is then measured by CT volumetry, in which serial liver scans at 1.0 cm intervals were taken and summed ($cm^3$).[14] The pre-operative PVE was applied in patients who would undergo major hepatectomy, in case the expected ratio of resected volume in the whole liver volume is more than 60% in patients without chronic liver disease, or more than 50% in patients with chronic hepatitis or cirrhosis by our protocol of liver resection. The study design was approved by the Ethics Review Board of our institution and a signed consent was obtained from each subject.

*Technique of portal vein embolization and evaluation*

Two approaches of access to right portal vein were a direct catheterization of ileocolic vein (n=4) and percutaneous transhepatic puncture (n=6) according to the coarse of the portal vein and the patient conditions. Portal pressures before and after PVE were measured to estimate the efficacy of embolization. Substances used for embolization in our series were 1g of absorbable gelatin sponge powder (Gelfoam®; Upjohn, Kalamazoo, MI, USA) and 5,000 units (5ml) of liquid thrombin (Sankyou Co., Tokyo, Japan) mixed in the contrast media. Permanent embolization materials have never been used. Embolization was completed when the entire right portal vein was completely occluded.

At 14 days after PVE, the hepatic volume of the unembolized lobe and embolized lobe (lobe to be resected) were reassessed by CT volumetry, and liver function tests including ICG R15 were also assessed again. Surgical resection of the liver was performed at 21 days after PVE.

*Measurement of serum HA*

Peripheral blood samples were collected in the early morning from each patient at the following time intervals: *1)* when the patient was at a stable condition during hospitalization, and *2)* 14 post-operative days after PVE. The blood sample was immediately centrifuged at 3,500G for 15 minutes, and 0.5 ml of serum was stored at -80℃. HA was assayed using the sandwich binding protein assay by SRL, Inc. (Tokyo, Japan) and the normal value was determined as less than 50 ng/ml. The serum HA level was compared with the changed volume of the embolized and unembolized liver at day 14 after PVE.

*Statistical analysis*

Data were expressed as a mean ± SD. Data of different groups were compared using one-way analysis of variance (ANOVA) and examined by Student's *t*-test. Correlations between two parameters were examined by calculating the Pearson's correlation coefficient. A two-tailed $P$ value $< 0.05$ was considered significant. The StatView Software for Windows, Version 5.0 (SAS Institute, Inc., Cary, NC) was used for all statistical analyses.

## Results

*Patient outcome after PVE*

Most of the patients had mild fever-up and mild elevation of serum AST and ALT levels (data not shown) for the first week and, however, recovered to the normal values at 14 days after PVE. No patients experienced severe complications or death. The value of ICG R15 did not significantly alter between pre- and post-PVE (11.4+/-4.9 % and 13.5+/-9.6 %, p=0.438). Therefore, all patients kept being scheduled to undergo right or extended right lobectomy of the liver.

*Relationship between serum HA level and the liver volume after PVE*

The mean serum HA concentration in all patients was 134 ± 108 ng/ml, and that in patients with chronic viral liver diseases (202±118 ng/ml) was significantly higher than in patients with normal and icteric liver (70±24 ng/ml) (p=0.048). The actual volume and its proportional ratio were shown in Table1. After PVE, the proportional ratio of the embolized right lobe was significantly decreased, and, on the other hand, the volume and the proportional ratio of the unembolized left lobe were significantly increased in patients with both non-CVLD and CVLD. Serum

HA levels after 2 weeks of PVE (296+/-216 ng/ml) tended to be greater than that before PVE (134+/-108 ng/ml) but not statistically significant (p=0.105). Before PVE, serum HA level significantly correlated with the value of ICG R15 (r=-0.509, p<0.05). However, on the other hands, serum HA level after 2 weeks of PVE did not correlate with the value of ICG R15 at the same stage (r=-0.358, p=0.760).

In Table 2, no significant correlation between the serum HA level before PVE and changes of the actual volume and the proportional ratio of embolized and unembolized lobes after PVE was observed in patients with either non-CVLD or CVLD.

**Table 1.** Changed volume of embolized and unembolized liver after PVE

a) Actual volume

| | Pre-PVE[2] ($cm^3$) | Post-PVE ($cm^3$) | Changed volume ($cm^3$) |
|---|---|---|---|
| Embolized lobe | 648+/-149 | 576+/-215[3] | -72+/-96 |
| Non-CVLD[1] (n=5) | 748+/-216 | 697+/-260 | -51+/-79 |
| CVLD (n=5) | 573+/-131* | 486+/-119[4]* | -87+/-107 |
| | | | |
| Non-embolized lobe | 428+/-167 | 534+/-170[3] | 106+/-67 |
| Non-CVLD (n=5) | 386+/-145 | 503+/-182[3] | 117+/-66 |
| CVLD (n=5) | 460+-180 | 558+/-164[3] | 98+/-69 |

b) Proportional ratio of lobe in the whole liver

| | Pre-PVE (%) | Post-PVE (%) | Changed ratio (%) |
|---|---|---|---|
| Embolized lobe | 60.5+/- 9.8 | 51.6+/- 9.8[3] | -8.9+/-5.5 |
| Non-CVLD (n=5) | 66.2+/- 3.3 | 57.9+/- 4.9[3] | -8.3+/-3.2 |
| CVLD (n=5) | 56.2+/- 10.9* | 46.9+/-10.0[3]** | -9.3+/-6.8 |
| | | | |
| Non-embolized lobe | 39.5+/-9.8 | 48.4+/- 9.8[3] | 8.9+/-5.5 |
| Non-CVLD (n=5) | 33.8+/-3.3 | 42.2+/- 4.9[3] | 8.3+/-3.2 |
| CVLD (n=5) | 43.8+-10.9* | 53.1+/-10.0[3]** | 9.3+/-6.8 |

1) chronic viral liver disease 2) portal vein embolization 3) p<0.01 vs pre-PVE 4) p<0.05 vs pre-PVE
*: p<0.05 vs non-CVLD **: p<0.01 vs non-CVLD

**Table 2.** Correlation between serum HA level and changed volume of embolized and unembolized liver after PVE

| | Correlation with actual volume | P value | Correlation with proportional ratio | P value |
|---|---|---|---|---|
| Embolized lobe | 0.043[2] | 0.910 | 0.106 | 0.779 |
| Non-CVLD[1] (n=5) | 0.062 | 0.930 | 0.632 | 0.292 |
| CVLD (n=5) | 0.503 | 0.434 | 0.317 | 0.642 |
| | | | | |
| Non-embolized lobe | -0.190 | 0.611 | -0.106 | 0.779 |
| Non-CVLD (n=5) | -0.022 | 0.976 | -0.632 | 0.292 |
| CVLD (n=5) | 0.463 | 0.478 | -0.317 | 0.642 |

1) chronic viral liver disease 2) correlation coefficient (r)

## Discussion

Measurement of serum hyaluronic acid level is a good index of liver function and has been recently used to evaluate indication or prediction of complications after liver resection and transplantation.[15,16] Serum HA levels were significantly high in patients with pre-cirrhotic and cirrhotic stage of chronic liver disease because higher HA level was caused by severe fibrosis and severe damage of endothelial cells in hepatic sinusoid.[6,8,11] Therefore, serum HA levels reflected the severity of liver disease. In the present study, the serum HA level was also higher in patients with chronic viral liver disease compared to those with normal liver and obstructive jaundice.

After major hepatectomy, regeneration of the remnant liver is the most important factor in the recovery of liver function and overcoming hepatic failure.[17] Ogata et al.[18] and our group[8] previously reported that the presence of high preoperative serum HA concentration was significantly associated with inhibition of hepatic regeneration after hepatectomy in patients with both normal liver and chronic liver diseases. Thus, it is possible to predict the regeneration capacity of the damaged liver before operation by measuring serum HA levels. As described above, nowadays PVE technique has been preferably applied to accelerate the hypertrophy of the remnant liver in order to prevent hepatic failure after major hepatectomy.[2,3] Although the regeneration activity of the liver after PVE may be based on any functional liver reserve, distinct predictive markers for regeneration by this technique have not been clarified so far. We hypothesized that the serum HA marker might be useful for this marker after PVE as well as in cases of hepatectomy. In the present study, the effect of PVE showing by changes of hepatic volume was significant and no patient had hepatic failure after liver resection. Changes of hepatic volume after PVE seemed not to be different between non-CVLD and CVLD. It has been reported that PVE does not lead severe liver damage.[2] Although the white blood cell counts, CRP, transaminase levels etc. transiently deteriorated after PVE, the liver functions were almost normalized after 2 weeks (data not shown in the present study). ICG R15 tended to worsen causing the decrease of clearance of ICG by the hepatic flow alteration but not significant change. The plan of right lobectomy or extended right lobectomy of the liver had not been changed at 2 weeks after PVE in all patients. Serum HA level did not significantly increase at 2 weeks after PVE in the present study, and, therefore, damage of endothelial cell function by PVE might not be severe. Serum HA level significantly correlated with the ICG R15 value as we reported previously,[8] and, however, HA level after PVE did not correlated with the ICG R15 value after PVE. By these results, hepatic blood flow alteration might not be associated with the clearance of HA and the damage of endothelial cell of hepatic sinusoid by PVE was minimized.

Unfortunately, the correlations between serum HA levels and changes of hepatic volume after PVE was not observed. In case of hepatectomy, the remnant liver may remarkably regenerate to maintain liver functions because the absolute hepatic volume decreases. While, in case of PVE, the embolized liver still maintain the arterial blood flow and hepatic functions,

and, therefore, the regeneration is probably slower compared to that after surgical resection. Furthermore, we speculate that the mechanism of regeneration of hepatic cells after PVE is different from that after major hepatic resection. Therefore, the serum HA level might not correlate with the changes of hepatic volume after PVE. By our results, it is indicated that the serum HA level before PVE is not a useful marker for prediction of the PVE affect contrary to the previous our results in cases after hepatectomy.[8]

In conclusion, we have demonstrated in the present study that serum HA level before PVE was not associated with the changes of embolized and unembolized lobe after PVE. It is clarified that the measurement of serum HA level before PVE is not useful to evaluate the efficacy of PVE technique.

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