Original paper

Clinical Significance of Measuring Urinary Sulfated Bile Acids in Adult Patients with Hepatobiliary Diseases.

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Running title: USBA correlates with liver dysfunction

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ABSTRACT

Background/Aims: Measurement of urinary sulfated bile acid (USBA) level is a simple urine test that reflects the degree of cholestasis in newborns. The aim of this study was to clarify the clinical significances of this test for liver diseases in adults.

Methodology: We examined the relationship between USBA level in a urine sample by enzymatic assay and clinical parameters and postoperative complications in 27 patients with hepatobiliary diseases who underwent surgical procedures between 2002 and 2007.

Results: Mean USBA in all patients before surgery was 39.8 ± 64.0 μmol/L (median value was 6.6). USBA level was increased in patients with cholestasis. USBA level was significantly correlated with serum total bile acid, total bilirubin level and serum hyaluronic acid level (r=0.850, 0.602 and 0.504, respectively) ($p<0.05$) and, furthermore, tended to be correlated with liver-uptake ratio (LHL15) by technetium-99m galactosyl human serum albumin (^{99m}Tc-GSA) scintigraphy and alanine aminotransferase level (r=-0.469 and 0.436, respectively but not significant). USBA level tended to be associated with postoperative uncontrolled ascites (p=0.050, not significant). Postoperative USBA level by day 7 was not changed; however, USBA level in patients with cholestatic diseases was decreased **Conclusions:** USBA is a simple and sensitive noninvasive test for cholestasis and also useful to predict postoperative uncontrolled ascites after hepatic resections.

KEYWORDS: Urinary sulfated bile acid; Cholestasis; Hepatectomy; Ascites

ABBREVIATIONS: urinary sulfated bile acid (USBA); technetium-99m galactosyl human serum albumin (^{99m}Tc-GSA); indocyanine green retention rate at 15 min (ICG R15)

INTRODUCTION

It is well known that the serum bile acid level is increased according to cholestasis in hepatobiliary diseases (1). This parameter is often used to investigate cholestatic diseases (2). Furthermore, sulfated bile acid refluxes into the serum under cholestatic status and is excreted in the urine (3). The urinary sulfated bile acid (USBA) level is thus increased and reflects the degree of cholestasis in liver diseases of animals and pediatric cholestatic diseases (4-7). Advantages of measuring USBA are that the test is noninvasive, it does not require blood samples, and it is sensitive for detecting cholestatic diseases at an early stage (4). Therefore, USBA has been used in animals and newborns. Additionally, there is no risk of transmitting infections via blood using this test. We measured USAB before and after operation in patients who had hepatobiliary diseases with the intent of clarifying the clinical significance of USBA by comparing several parameters.

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METHODOLOGY

Patients

The subjects were 27 patients with hepatobiliary diseases who underwent surgical resections in the Division of Surgical Oncology, Department of Translational Medical Sciences, Nagasaki University Graduate School of Biomedical Sciences between 2003 and 2007. They included 15 males and 12 females with a mean age of 67.2±8.5 years (±SD, range, 50-80 years). Injured liver diseases included 10 hepatocellular carcinomas, 2 intrahepatic cholangiocarcinomas, 6 metastatic liver tumors originating from colorectal carcinoma, a gall bladder carcinoma, 6 bile duct carcinomas and 2 benign liver tumors. The background liver diseases included chronic viral liver diseases in 10 (including 3 cases of cirrhosis caused in 4 by hepatitis viral B and 6 by hepatitis viral C) and obstructive jaundice in 7 patients and normal liver function in 10.

In our hospital, the volume of liver to be resected is estimated before surgery based on the results of indocyanine green retention rate at 15 min (ICG R15) using Takasaki's formula (8) and liver activity at 15 minutes by technetium-99m galactosyl human serum albumin scintigraphy (LHL15) in case of hepatectomy (9). In case of biliary diseases with obstructive jaundice, the operation was postponed until the total bilirubin level was under 2 mg/dL. Hepatic resection was performed in 23 patients including limited resection in 8 patients, segmentectomy in 5, and lobectomy in 10. Four other patients underwent pylorus preserving pancreaticoduodenectomy. Post-operative complications included persistent ascites in 5 (representing massive ascites even under treatment with diuretics for more than two weeks), hepatic failure in 1 (represented by total bilirubin > 3 mg/dL at day 28 postoperatively), and intra-abdominal infection in one patient. There

was no hospital stay in the present series. The study design was approved by the Ethics Review Board of our institution and a signed consent for measuring USBA was obtained from each subject.

Measurement of USBA

Urine samples were collected in the early morning from each patient before surgery when the patient was in a stable condition during hospitalization. In patients with obstructive jaundice and hyperbilirubinemia, samples were not collected until total bilirubin level was improved at less than 2 mg/dL by biliary drainage. The urine sample was stored at -20°C before USBA assay (4, 10). USBA was measured by a commercially available kit, UBASTEC by Marukin Bio, Inc. (Kyoto, Japan), utilizing direct enzymatic assay. (10) USBA value was corrected by creatinine clearance and the normal value was determined as less than 10 μmol/g creatinine by laboratory data. Platelet count, prothrombin activity, and serum level of total bilirubin, alanine aminotransferase, total cholesterol or hyaluronic acid were examined by blood test.

Statistical analysis

Data were expressed as mean ± SD. Data of different groups were compared using one-way analysis of variance (ANOVA) and examined by Student's *t*-test or Dunnet's multiple comparison test. Correlations between two parameters were examined by calculating the Pearson's correlation coefficient. A two-tailed P value < 0.05 was considered significant. Statistical analyses were performed using the computer software STATISTICA™ (StatSoft, Tulsa, OK).

RESULTS

The preoperative mean and median concentrations of USBA in all patients were 39.8±64.0 and 6.6μmol/g creatinine (ranging from 0.8 to 285.7μmol/g creatinine), respectively. The USBA level in these patients was not associated with gender or Child-Pugh classification (**Table 1**). USBA was significantly increased in patients with obstructive jaundice; however, USBA in chronic viral hepatitis or cirrhosis was similar to that in normal liver. The correlation between USBA and patient age or liver function tests is shown in **Table 2**. USBA was not associated with patient age. USBA was significantly correlated with the results of serum total bile acid level, total bilirubin level, and serum hyaluronic acid level. Furthermore, USBA tended to be correlated with alanine aminotransferase level and liver-uptake ratio (LHL15) by technetium-99m galactosyl human serum albumin (^{99m}Tc-GSA) scintigraphy.

Figure 1 shows postoperative changes in mean USBA after hepatic resection. USBA level decreased at day 1 after hepatectomy and was maintained at day 7. **Figure 2** shows postoperative changes of mean USBA in each liver disease. Although USBA level was increased in patients with obstructive jaundice, this level was immediately decreased at day 1. Postoperative USBA levels were not significantly different between liver diseases. Preoperative USBA level in patients with postoperative uncontrolled ascites tended to be higher than that in patients without ascites (**Table 1**). **Figure 3** shows postoperative changes of mean USBA in patients with or without uncontrolled ascites after operation. USBA level was significantly increased at day 3 in patients with uncontrolled ascites, however, which was immediately improved at day 7.

DISCUSSION

Although sulfated bile acid in liver cells is excreted into the bile, it is refluxed into the serum under cholestatic conditions (1-3). Sulfated serum bile has high water solubility and is immediately excreted in the urine as USBA (11). Measurement of USBA is expected to be a useful test for diagnosis and analysis of cholestasis in hepatobiliary diseases. As in previous reports (4-7), USBA was significantly correlated with serum bile acid level in our series. Obatake et al. (4) reported that USBA was increased in neonatal patients with cholestasis such as biliary atresia. They described that USBA still increased under conditions of cholangitis as well. In the present study, USBA was higher in patients with obstructive biliary diseases. In these patients, the high bilirubin level was improved by biliary drainage; however, USBA was still higher. Although USBA was correlated with higher serum bilirubin level as the present result, this parameter might be sensitive for congestion of bile or inflammation of bile ducts. As our result indicated, USBA level in cholestatic diseases dramatically decreased and immediately improved after hepatectomy. Release of biliary congestion might cause this improvement of USBA. Although Obatake et al. reported that USBA was not correlated with any liver function tests and was an independent parameter to diagnose biliary atresia (4), USBA was also correlated with serum hyaluronic acid level or other liver dysfunctions in adult patients of the present study. Particularly, our previous study indicated that hyaluronic acid level or LHL15 by ^{99m}-Tc GSA liver scintigraphy were useful functional parameters to estimate hepatocellular damage and to predict postoperative hepatic complications (12, 13). USBA might be related to total parenteral nutrition (TPN)-associated cholestasis (4, 14). In our series, some patients were

examined under the condition of TPN. However, no significant relationship could be observed because of the small number of patients (n = 2) (not shown in result).

As USBA level was associated with hyaluronic acid level or LHL15 as described above, it is possible that USBA was able to predict or monitor postoperative complications. As expected, preoperative USBA or USBA at day 3 was related to uncontrolled ascites. This complication was likely caused by cellular damage of the liver. USBA may be a sensitive hepatic marker to predict postoperative liver damage. To our knowledge, the possibility of using this marker has not been reported. In the present series, hepatic failure or intraabdominal infection was observed in only one patient. To clarify the usefulness of USBA to predict postoperative hepatic complications, a larger number of patients with severe hepatic complications after hepatectomy must be examined.

Some advantages of testing USBA in comparison with measuring serum bile acid level include: 1) Non-invasiveness because no blood sampling is necessary. USBA test can be used in infants or obese patients. 2) Sampling is easy and, therefore, it can be applied for screening tests in a large number of subjects. 3) Lower risk of transmitting infections via blood for both patients and investigators in the laboratory compared to blood sampling (4). The disadvantages of USBA test at present are a higher cost and lack of rapid report. These disadvantages will be resolved with improvement of the system in the near future.

In conclusion, we have demonstrated that USBA was significantly correlated with biliary cholestasis in hepatobiliary diseases, which were immediately improved by the complete resection of obstructive portions. USBA was correlated with serum bile acid

level and some liver functions. USBA is a simple and sensitive noninvasive test for cholestasis and is also useful to predict postoperative uncontrolled ascites by the hepatic damages after hepatic resections.

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FIGURE LEGENDS

FIGURE 1 Serial changes in USBA concentrations after hepatectomy. Data are expressed as means ± SD. Preop., preoperative.

FIGURE 2 Serial changes in USBA concentrations in each liver disease after hepatectomy. Preop., preoperative. Solid line shows the normal liver, long broken line shows the obstructive jaundice, small dotted line shows chronic viral hepatitis and long chain line shows cirrhosis. *: Significant difference between USBA concentration preoperatively and on postoperative day 3 in patients with obstructive jaundice.

FIGURE 3 Serial changes in USBA concentrations in patients with or without uncontrolled ascites after hepatectomy. Preop., preoperative. Solid line shows negative ascites, and the long broken line shows positive ascites. *: Significant difference between USBA concentration preoperatively and on postoperative day 3 in patients with uncontrolled ascites.

TABLE 1 Relationship between USBA Concentrations and Patient Demographics and Postoperative Complication.

	USBA level (μmol/g creatinine)
Gender	
Male (n=15)	10.0±10.5
Female (n=12)	31.1±37.0
Background liver disease	
Normal (n=10)	18.4±38.7
Obstructive jaundice (n=7)	73.4±52.1*
Chronic viral hepatitis (n=7)	9.4±6.3
Cirrhosis (n=3)	18.4±12.5
Child-Pugh classification	
A (n=25)	9.9±9.9
B (n=2)	16.1±20.2
Postoperative uncontrolled ascites	
No (n=22)	6.9±8.0
Yes (n=5)	15.9±11.1#

Data are expressed as means ± SD. Data of different groups were examined by Student's *t*-test and Dunnet's multiple comparison test.

*; P<0.05 vs. chronic viral hepatitis. #; p=0.50

TABLE 2 Correlation between USBA Concentrations, and Patient Age or Liver Function Tests.

	correlation coefficient (r)	***P* value**
Age	-0.300	0.281
Serum total bile acid (μg/ml)	0.850	<0.01
Total bilirubin (mg/dL)	0.602	0.014
Alanine aminotransferase (IU/L)	0.436	0.092
Platelet count (/mm^3)	0.256	0.338
Total cholesterol (mg/dL)	-0.039	0.885
Prothrombin activity (%)	-0.057	0.833
Hyaluronic acid (ng/dL)	0.542	0.030
ICGR15 (%)	0.323	0.240
LHL15	-0.469	0.078

ICGR15: indocyanine green retention rate at 15 minutes

LHL15 indicates Hepatic uptake ratio by ^{99m}Tc-GSA

FIGURE 1

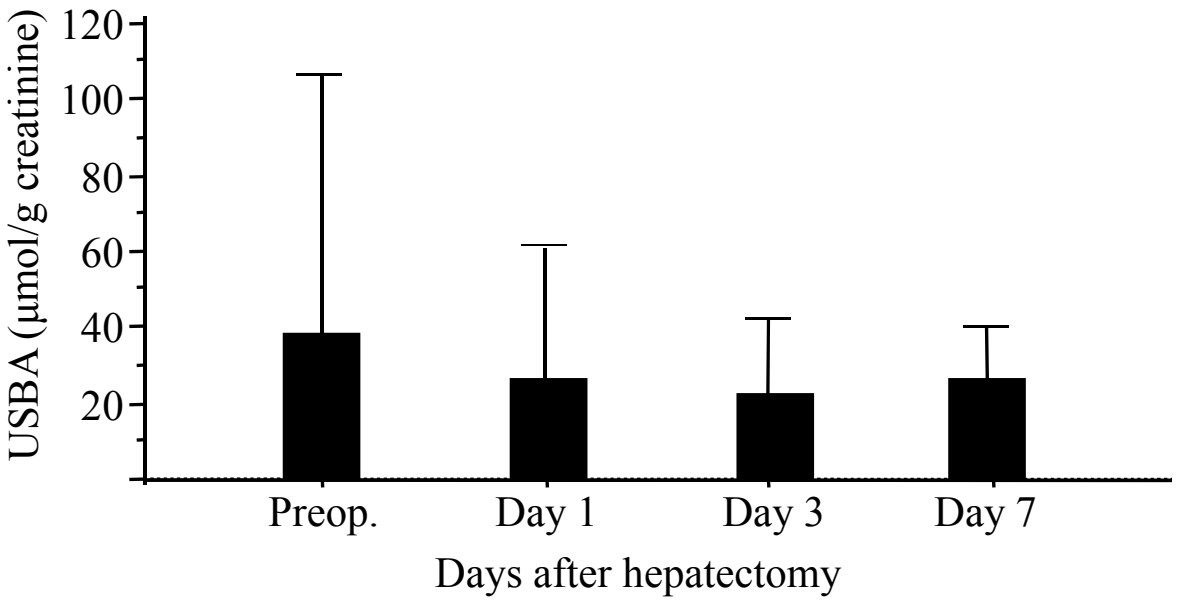

USBA (µmol/g creatinine)
120
100
80
60
40
20
Preop.
Day 1
Day 3
Day 7
Days after hepatectomy

FIGURE 2

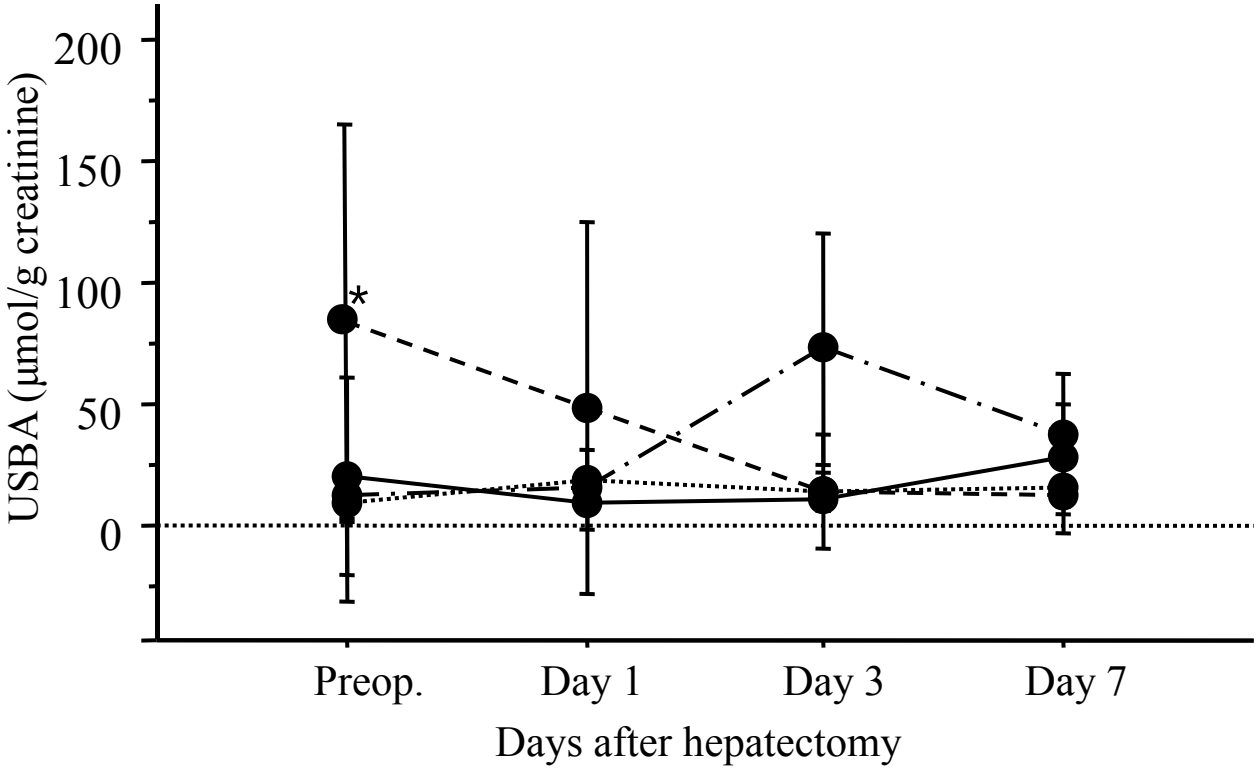
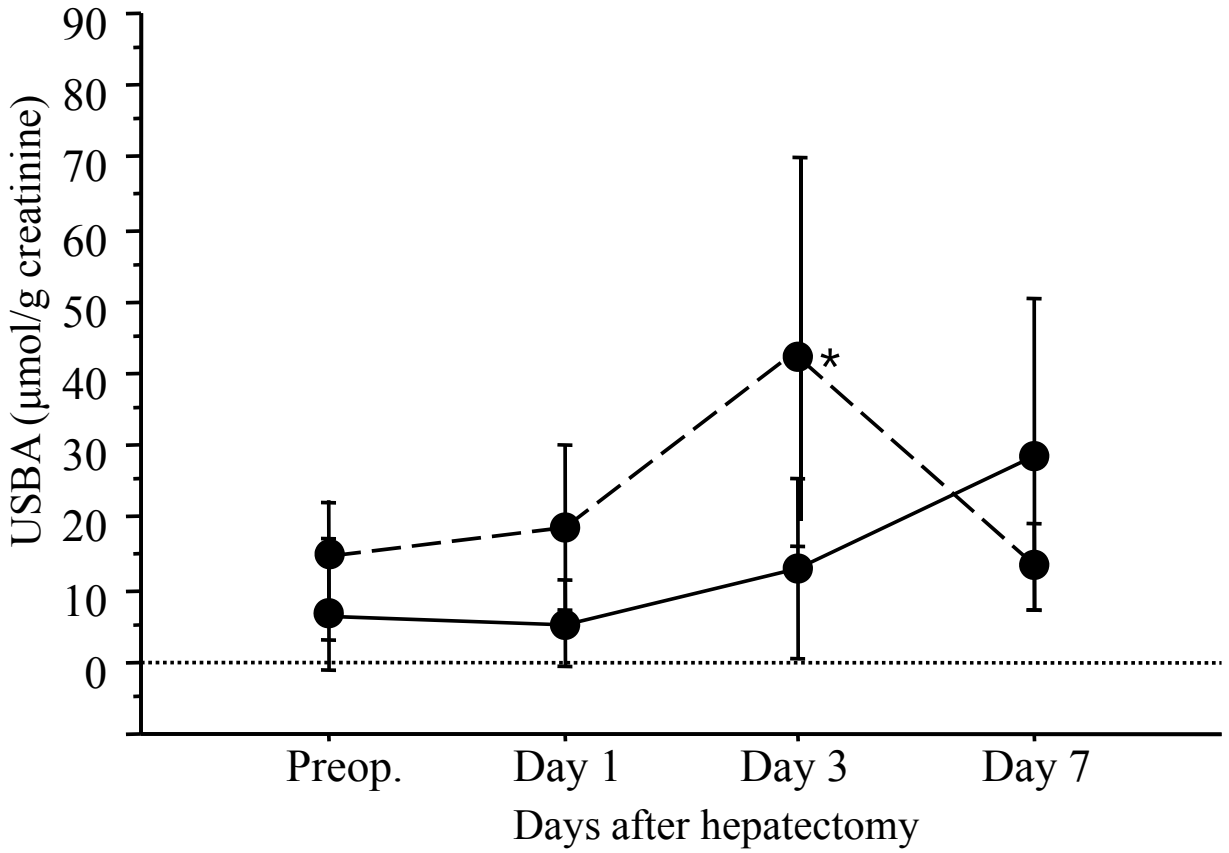

200
150
100
50
0
USBA (μmol/g creatinine)
*
Preop.
Day 1
Day 3
Day 7
Days after hepatectomy

FIGURE 3


90
80
70
60
50
40
30
20
10
0
USBA (μmol/g creatinine)
*
Preop.
Day 1
Day 3
Day 7
Days after hepatectomy