นิพนธ์ต้นฉบับ

Serum bile acids in hepatitis B carriers.

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Total serum bile acids were measured in HBsAg carriers by an enzymatic colorimetric method using 3α-hydroxysteroid dehydrogenase (3α-HSD). Twenty carriers had mild nonspecific change or chronic persistent hepatitis (CPH), and 14 had chronic active hepatitis (CAH). The sensitivity and specificity of fasting total serum bile acids in the diagnosis of CAH (when 6 micromol/l was used as the cut-off point) was 100% and 90%, respectively. The 1-2-and 3-hour postprandial total serum bile acids (at the cut-off levels of 15, 22 and 15 micromol/l) had sensitivity of 79-93% with specificity of 85-90% whereas routine liver function tests provided sensitivity of 50-79% and specificity of 45-95%, respectively.

We conclude that the estimation of fasting total serum bile acids appears to be beneficial in the diagnosis of chronic active hepatitis in hepatitis B carriers suspicious of having chronic liver disease.

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บุษบา มาตระกูล, พงษ์เชษฐ์ เบศรภิญโญวงศ์, พงษ์พีระ สุวรรณกูล, พินิจ กุลละวณิชย์, สัจพันธ์ อิศรเสนา. ระดับของกรดน้ำดีในซีรัมของผู้เป็นพาหะของไวรัสตับอักเสบบี. จุฬาลงกรณ์เวชสาร 2537 ธันวาคม; 38(12): 743-751

ได้ทำการตรวจหาระดับของกรดน้ำดีในซีรัมของผู้เป็นพาหะของไวรัสตับอักเสบบี จำนวน 34 ราย โดยวิธี enzymatic (3α-hydroxysteroid dehydrogenase) ผู้เป็นพาหะ 20 ราย มีพยาธิสภาพที่ตับแบบไม่รุนแรง (non-specific หรือ chronic persistent hepatitis) และ 14 ราย มีพยาธิสภาพแบบรุนแรง (chronic active hepatitis) จากการศึกษาพบว่าความไวและความจำเพาะของระดับกรดน้ำดีในซีรัมเมื่ออดอาหาร (เมื่อใช้ระดับ 6 ไมโครโมล/ลิตร เป็นระดับตัดสิน) เท่ากับ 100% และ 90% ตามลำดับ สำหรับระดับกรดน้ำดีในเลือดหลังรับประทานอาหาร 1,2 และ 3 ชม. (เมื่อใช้ระดับ 5,22 และ 15 ไมโครโมล/ลิตร เป็นระดับตัดสิน) จะมีความไวเท่ากับ 50-79% และความจำเพาะ 45-95% ตามลำดับ จากการศึกษานี้แสดงว่าการตรวจหาระดับของกรดน้ำดีในซีรัมเมื่ออดอาหารมีประโยชน์ในการวินิจฉัย chronic active hepatitis ในผู้เป็นพาหะของไวรัสตับอักเสบบี

Hepatitis B viral infection is one of the major health problems in Thailand. About ten percent of the population are hepatitis B carriers who may later develop chronic hepatitis, cirrhosis or hepatoma.[1,2] Since conventional biochemical tests are mostly of little use in defining various forms of chronic hepatitis, liver biopsy is required for definite diagnosis, severity, and progression of the disease. Estimation of serum bile acids has been considered to be a more sensitive indicator of liver disease than conventional liver function tests.[3-7] Serum bile acids were significantly increased in chronic hepatitis in comparison to control subjects and the increases were more evident and constant in CAH.[5, 8-10] Serum bile acids levels were found to be related to the histological features of a group of patients with chronic hepatitis, so that serum bile acids were proposed as an indicator for selecting patients for liver biopsy[10] or as an alternative to repeating liver biopsies.[5]

In this study the diagnostic value of fasting and postprandial serum bile acids were compared with conventional liver function tests in hepatitis B surface antigen carriers with different histologic features.

Materials and Methods

Control subjects

Twelve healthy volunteers were studied. These were 6 male and 6 female hospital staff members aged 25 to 40 years. None of them had previous hepatobiliary disease. They were negative for HBsAg. All had normal liver function tests, and ultrasound examination of the liver and biliary tract was also normal.

Patients

Thirty-four HBsAg carriers with no evidence of intestinal or hepatobiliary diseases were studied. They had no history of taking drugs or significant amounts of alcohol. These patients were diagnosed as having nonspecific change or chronic hepatitis, with or without cirrhosis, by histological findings of liver biopsies.

Procedure

Blood specimens were obtained in the morning after fasting for 12 hours. Thereafter, the subjects were given a 450-kcal test meal containing 45% carbohydrate, 20% protein and 35% fat. Three postprandial blood samples were drawn at one-hour intervals. After clotting, the serum was separated and stored at -20°c until analyzed.

Bile acids determination

Serum bile acids were estimated by the enzymatic colorimetric 3α-hydroxysteroid dehydrogenase method using an Enzabile kit (Nyegaard, Oslo, Norway).[11] This method is based upon the conversion of 3α-hydroxy bile acids to 3-keto derivatives by 3α-hydroxy-steroid dehydrogenase (3α-HSD) in the presence of NAD. The NADH formed reacts with nitrobluetetrazolium salt (NBT) under the catalytic influence of diaphorase to give a blue formazan derivative. The amount of formazan produced is measured photometrically at 540 nm using a Photometer 4020 (Boehringer Mannheim GmBH, Mannheim, Germany). Nyegaard Bile Acid Standard and Seronorm Lipid Control Serum were used as the standard and control.

Bilirubin, AST, ALT, total protein and albumin were measured by means of routine laboratory methods.

Statistical methods

The results were expressed as mean ± standard deviations. Tests of significance between groups of data were carried out using unpaired Students't-tests, the Fisher exact test and ANOVA. A Receiver Operating Characteristics Curve was used to identify the cut-off point for the level of serum bile acids in the differentiation of CAH from CPH and non-specific changes.

Results

The 34 patients were classified into 2 groups by histological features of their liver biopsy: 20 non-CAH (mild nonspecific histological change and chronic persistent hepatitis) and 14 CAH (with or without cirrhosis). The characteristics of the 34 carriers are shown in Table 1. There was no significant difference between the non-CAH and CAH groups regarding age, sex, body weight and height. In CAH, symptoms and signs of liver disease were found in 50% and 70% of the patients.

Table 1. Characters of HBsAg positive subjects.

	non CAH (n=20)	CAH (n=14)
age (years)	26.4 ± 8.7	32.9 ± 13.2
male	16	11
female	4	3
body weight (kg)	48.9 ± 5.4	54.5 ± 9.2
hight (cm)	162.4 ± 6.7	163.6 ± 8.2
history of jaundice	5	7
sign (positive)	4	10
symptom (positive)	0	7

Table 2 shows the results of routine liver function tests in non-CAH and CAH groups. Only globulin in the CAH group was significantly higher than in the non-CAH group.

Table 2. Results of routine liver function tests (mean ± SD).

	non CAH (n=2)	CAH (n=14)
Bilirubin, direct (mg/dl)	0.17 ± 0.08	0.71 ± 0.95
Bilirubin, total (mg/dl)	1.01 ± 0.33	1.81 ± 1.41
AST (U/L)	29.90 ± 14.11	151.43 ± 70.84
Albumin (g/dl)	4.04 ± 0.65	3.61 ± 0.72
Globulin (g/dl)	2.32 ± 0.56	3.22 ± 0.95 *

*t-test $P < 0.005$

Figures 1,2,3,4, demonstrate Receiver Operating Characteristics (ROC) curves of fasting and postprandial serum bile acids. From the ROC curves, the levels of serum bile acids giving best specificity and sensitivity of fasting in 1-2-and 3-hour postprandial samples were 6,15, 22 and 15 micromol/l, respectively.

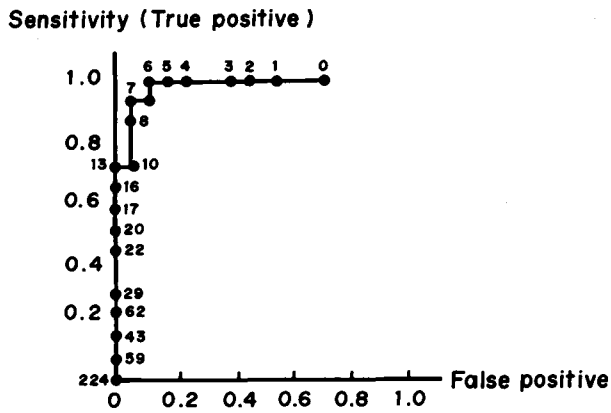

Figure 1. FSBA receiver operating characteristics curve (Fasting serum bile acid).

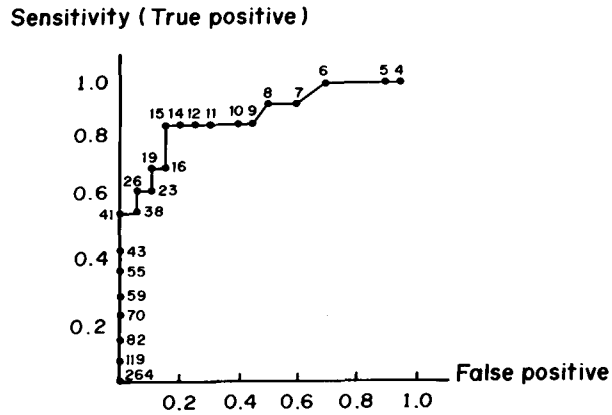

Figure 2. SBA1 receiver operating characteristics curve (1 hour-post pandrial serum bile acid).

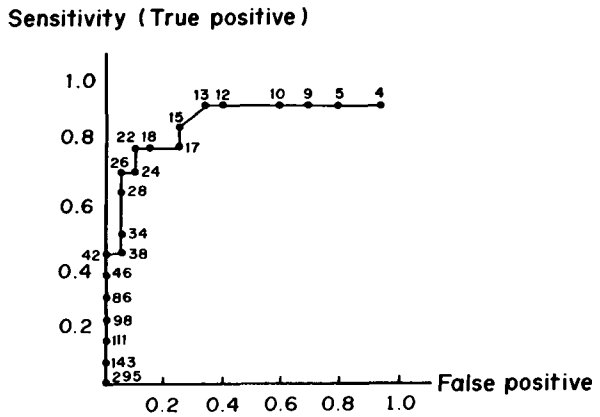

Figure 3. SBA2 receiver operating characteristics curve (2 hour-postpandrial serum bile acid).

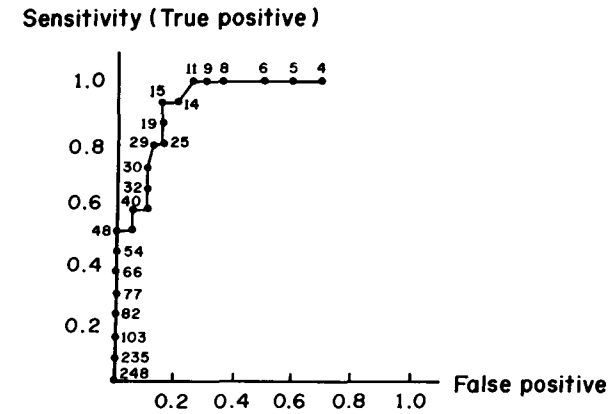

Figure 4. SBA3 receiver operating characteristics curve (3 hour-post pandrial serum bile acid).

The results of fasting and postprandial serum bile acid levels in normal, non-CAH and CAH subjects are shown in Table 3. In 3 normal subjects, the serum bile acid levels ranged between 0 and 6 micromol/l. The fasting serum bile acid levels were above the normal ranges in all 14 cases of CAH but in only 2 of the 20 cases of non-CAH. The postprandial serum bile acid levels of the CAH subjects were significantly higher than those of the other 2 groups.

Table 3. Results of serum bile acids (mean ± SD).

	Normal subjects (n=12)	non-CAH (n=20)	CAH (n=14)
FSBA (micromol/l)	2.50 ± 1.45	2.90 ± 3.32	39.70 ± 55.45[a,b]
SBA 1(micromol/l)	7.50 ± 3.09	11.50 ± 8.72	60.43 ± 66.42[a,b]
SBA 2(micromol/l)	8.42 ± 5.18	13.90 ± 8.62	69.57 ± 79.69[a,b]
SBA 3(micromol/l)	8.42 ± 3.89	11.60 ± 12.69	77.64 ± 78.30[a,b]

ANOVA a $P < 0.05$ compared with normal subjects
ANOVA b $P < 0.05$ compared with non-CAH subjects
FSBA = Fasting serum bile acids
SBA 1= 1-hr postprandial serum bile acids
SBA 2= 2-hr postprandial serum bile acids
SBA 3= 3-hr postprandial serum bile acids

The sensitivity and specificity of routine liver function tests in diagnosing CAH were 50-79% and 45-95%, respectively (Table 4). Serum bile acids had 79-93% sensitivity and 85-90% specificity in diagnosing CAH when fasting. 1-2-and 3-hour postprandial serum bile acid levels of 6,15,22 and 15 micromol/l, respectively were used as the cut-off points (Table 5).

Table 4. Sensitivity and specificity of routine liver function tests in the diagnosis of chronic active hepatitis.

	Sensitivity	Specificity	P value
Bilirubin, direct > 0.3 mg/dl	0.57	0.95	0.001
Bilirubin, total > 1.0 mg/dl	0.79	0.45	0.11
AST/ALT > 1	0.50	0.85	0.035
Albumin < 3.5 g/dl	0.50	0.85	0.04
Globulin > 3.0 g/dl	0.57	0.84	0.017
Alb/Glob < 1	0.50	0.94	0.005

Table 5. Sensitivity and specificity of serum bile acids in the diagnosis of chronic active hepatitis.

	Sensitivity	Specificity	P value
FSBA > 6 micromol/l	1	0.90	0.000001
SBA 1> 15 micromol/l	0.86	0.85	0.0002
SBA 2> 22 micromol/l	0.79	0.90	0.0002
SBA 3> 15 micromol/l	0.93	0.85	0.00004

Discussion

In the past, use of serum bile acid as a diagnostic test was limited because of the technical difficulty in the analysis. The availability of a simple method has recently made serum bile acid an additional test to the conventional liver function tests used in several laboratories. The determination of total serum bile acids by an enzymatic method is easily performed and has an acceptable accuracy and precision. Our reference values for fasting serum bile acids were close to those obtained in other studies by enzymatic methods.[12-15]

Serum bile acids are elevated in most patients with chronic liver diseases due to impaired hepatic bile acid removal secondary to hepatocelluler dysfunction or portal systemic shunting.[16-17] Serum bile acid is of relatively low value in screening for patients who might have liver disease or in detecting mild liver disease,[9,18,19] but it is useful in identifying patients having significant liver disease in whom conventional tests may be normal or borderline.[15] It has been used to distinguish mild from severe chronic liver disease[8,10] and it has also proven to be a clinically useful prognostic index in cirrhosis.[20]

Measurement of individual bile acids and their conjugates has been reported to enhance the diagnostic value of other liver function tests.[21] Total bile acid and chenodeoxycholic acid levels after ursodeoxycholic acid administration were also suggested to be useful in the differentiation of chronic hepatitis from cirrhosis.[22]

The simplest bile acid measurement is the total bile acid concentration, either in the fasting state or after a meal. Our study showed that fasting and 3-hour postprandial serum bile acid levels in all 14 cases of CAH were elevated. When fasting, serum bile acid levels of 6 umol/1 was used as the cut-off point, and 100% sensitivity and 90% specificity were achieved. Postprandial samples provided less sensitivity and specificity. Eventhough some reports suggested that 2-hour postprandial values were more sensitive indicators of hepatobiliary disorders than fasing values when enterohepatic circulation of bile acids was taken into consideration.[6,13,14,23] Others did not find significant differences.[24,25]

Since serum bile acids test is highly specific for the presence of significant liver disease (except in the presence of bacterial contamination of the small intestine), but less sensitive in detecting early acute or mild liver diseases, on the basis of our findings, we would like to introduce fasting serum blie acid estimation as a routine laboratory test for the diagnosis of chronic active hepatitis in chronic hepatitis B carriers. Further studies using larger numbers of chronic hepatitis patients should be evaluated to determine the consistency of this findings.

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