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Profiles of Serum Bile Acids in Liver Diseases

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Cholylglycine (CG) and sulfolithocholylglycine (SLCG) in fasting and postprandial serum were determined in patients with liver diseases by radioimmunoassay. In normal controls, fasting CG and SLCG were 54.67±68.66ng/100ml and 16.61±12.84ng/100ml respectively, while postprandial CG and SLCG were 61.21±37.29ng/100ml and 21.95±15.9ng/100ml respectively.

In liver disease, serum bile acids were elevated. The greatest increase was found in acute viral hepatitis but moderate or slight increase was also found in chronic active hepatitis, liver cirrhosis, and hepatoma. Fasting bile acids seem to be a sensitive index for hepatocellular dysfunction but not for differential diagnosis of liver diseases.

In liver diseases except hepatoma, fasting CG and SLCG correlated significantly with total bilirubin, albumin, GOT, GPT, and alkaline phosphatase but not with cholesterol.

Insignificant elevation of bile acids was found postprandially in patients with liver diseases as well as normal controls and postprandial bile acids were not more sensitive than fasting ones.

Key Words: *Cholylglycine. Sulfolithocholylglycine. Glutamic oxalo-acetic transaminase. Glutamic pyruvate transaminase*

INTRODUCTION

Altered serum bile acids concentration has been known to occur in patients with liver diseases.[1-13]

Bile acids are formed in the liver from cholesterol, excreted via bile into the intestine, where they are reabsorbed into portal circulation and returned to the liver. Under normal circumstances serum bile acids depend closely on this enterohepatic circulation. Thus, serum bile acid levels reflect the amount of those escaping extraction from the portal blood. In liver disase, serum bile acids depend on the relationship between the decreased hepatic extration efficiency or reduced functional hepatocyte mass and portal-systemic shunting.[7]

Estimations of serum bile acids may provide a useful means for the detection of liver disease such as viral hepatitis, mild liver injury by hepatotoxic drugs,[7] and for further evaluation of patients with chronic active hepatitis[8] who were treated successfully.

It has also been suggested that measuring serum bile acids two hours after a meal was the method of choice for detecting hepatobiliary disease rather than conventional liver function testing.[3-5] Some workers have advocated the use of the cholic: chenodeoxycholic acid ratio as well as a intravenous ^{14}C cholylglycine tolerance test in differential diagnosis of liver disease.[9,10]

To evaluate the diagnostic significance of bile acids in liver diseases, fasting and postprandial serum bile acid concentrations were measured in patients with acute and chronic liver diseases. In addition, the diagnostic value of serum bile acids were compared with conventional liver function tests.

MATERIALS AND METHODS

Eleven healthy subjects with no history of hepatic or gastrointestinal disease and with normal biochemical tests were chosen as controls. Fifty-three patients with liver disease were studied; 19 with acute viral hepatitis; 9 with chronic active

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hepatitis; 14 with liver cirrhosis; and 11 with hepatoma. Diagnosis was made on the base of clinical symptoms, liver function tests and/or liver biopsy.

The fasting serum samples were obtained in the morning after overnight fasting. Two hours after meat, second samples were taken. The sera were kept frozen at −20°C until tested.

Cholylglycine (CG) and sulfolithocholylglycine (SLCG) were measured by radioimmunoassay using ^{125}I-CG RIA and ^{125}I-SLCG RIA Kits (Abbott Laboratories. Abbott Park, North Chicago, Illinois).

Routine liver function tests were performed by conventional methods.

Data are presented as mean ±SD (standard deviation of the mean). Significances of differences were assessed with Student's paired t-test. Correlations between variables were determined by calculating the correlation coefficient, r.

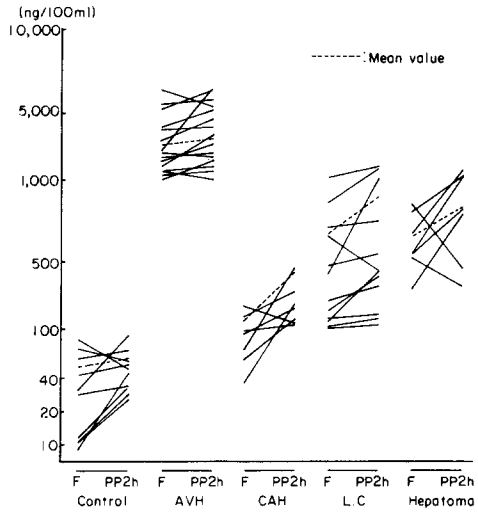

Fig. 1. *Fasting and postprandial CG in liver disease.*

Table 1. The level of serum bile acids in liver diseases

Diagnosis (No.)	CG(ng/100ml)		SLCG(ng/100ml)	
	Fasting	PP 2hr	Fasting	PP 2hr
Control (11)	54.67± 68.66	61.21± 37.29	16.61± 12.84	21.95± 15.98
AVH (19)	3,013.68±2,580.61	3,434.87±2,651.08	282.26±170.05	269.39±159.83
CAH (9)	169.31± 171.80	457.56± 457.62	94.34± 94.89	136.9 ±135.38
L.C (14)	680.12± 984.73	909.25± 974.00	101.41± 59.20	117.4 ± 64.50
PHC (11)	664.61± 559.75	845.52± 598.99	160.99±253.14	112.75± 52.81

AVH: acute viral hepatitis L.C: liver cirrhosis CAH: chronic active hepatitis
No: number of cases PHC: primary hepatocellular carcinoma

RESULTS

1. Serum CG and SLCG levels (Table 1, 2, Figure 1, 2)

In controls fasting CG and SLCG were 54.67±68.66ng/dl and 16.61±12.84ng/dl respectively. In both acute and chronic liver diseases fasting CG and SLCG were increased and the greatest increase was found in acute viral hepatitis; CG was 3013.68±2580.61ng/dl; and SLCG, 282.26±170.05ng/dl.

Two-hour postprandial CG and SLCG were higher than fasting level in controls and all liver diseases except hepatoma, but the increase had no statistical significance. The highest increase of two-hour postprandial ones was found in patients with chronic hepatitis. In patients with hepatoma, two-hour postprandial SLCG were frequently lower than fasting levels. Among patients with normal fasting bile acids, 2 with chronic active hepatitis and one with hepatoma had elevated two-hour postprandial CG and 2 with chronic active hepatitis had elevated two-hour postprandial SLCG. In these five instances, no statistical difference was demonstrated.

Table 2. Ratio of fasting and postprandial bile acids in liver diseases

	CG	SLCG
	PP 2hr/fasting	PP 2hr/fasting
Control	1.98±1.11	1.38±0.45
AVH	1.16±0.43	1.13±0.21
CAH	3.3 ±2.23	1.71±1.04
L.C	2.43±3.36	1.18±0.39
PHC	1.36±0.68	1.13±0.39

2. Comparison of serum bile acids with conventional liver function test (Table 3-1, 3-2)

Fasting CG correlated significantly with alkaline phosphatase and GOT in acute viral hepatitis, GOT in chronic active hepatitis, and GOT, GPT and bilirubin in liver cirrhosis respectively, but there was no significant correlation between fasting CG and other liver function tests in hepatoma. Fasting SLCG also correlated significantly with albumin and GOT in acute viral hepatitis, GOT and GPT in chronic active hepatitis and albumin in liver cirrhosis, respectively. Therefore, in acute and chronic liver diseases, fasting serum bile acids tended to correlate similary with transaminase levels. In addition, although bilirubin was normal, both fasting CG and SLCG were elevated in 5 patients with chronic active hepatitis and 7 patients with liver cirrhosis.

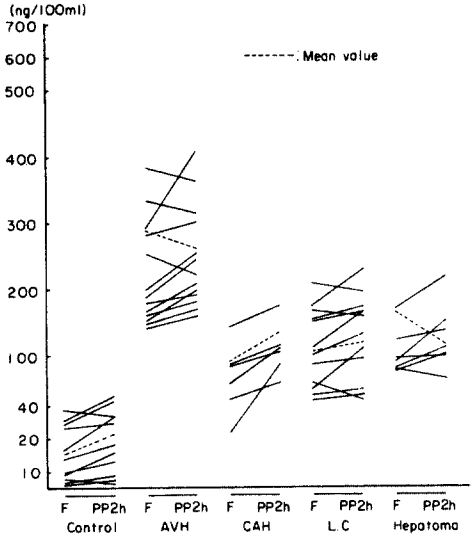

Fig. 2. *Fasting and postprandial SLCG in liver diseases.*

Table 3-1. Correlation of fasting CG with other tests in liver diseases

Relationship	AVH	CAH	L.C	PHC
	r	r	r	r
Albumin	0.03	0.38	−0.15	−0.04
Alk. P	0.80**	−0.33	0.21	−0.04
GOT	0.50*	0.67*	0.85**	0.16
GPT	0.35	0.59	0.89**	−0.20
Total Bil	0.36	0.34	0.94**	0.50
Cholesterol	0.02	−0.18	−0.28	−0.30

r: a coefficient of correlation * $p<0.05$
p: probability of chance association ** $p<0.001$

Table 3-2. Correlation of fasting SLCG with other tests in liver diseases

Relationship	AVH	CAH	L.C	PHC
	r	r	r	r
Albumin	0.56**	0.50	0.50	−0.09
Alk. P	0.24	−0.54	−0.44	0.06
GOT	0.40	0.93**	0.05	−0.23
GPT	0.51*	0.85**	0.05	−0.21
Total Bil	0.18	0.60	−0.06	−0.16
Cholesterol	−0.25	−0.20	0.38	0.10

r: a coefficient of correlation * $p<0.05$
p: probability of chance association ** $p<0.001$

DISCUSSION

Bile acids have a uniquely efficient enterohepatic circulation. The total body pool of primary and secondary bile acids ranges from 2 to 5g, and undergoes enterohepatic circulation two to three times per meal, or six to ten times daily.[16] The liver extracts bile acids incompletely from portal venous blood during a single passage, but the first-pass hepatic extraction of conjugated or unconjugated bile acids is highly efficient.[17] Approximately 70 per cent of the dihydroxy and 90 per cent of the trihydroxy bile acids are extracted, and it results in low concentrations in peripheral blood.

Monohydroxy bile acids such as lithocholate, as compared with di- and trihydroxy bile acids, is preferentially sulfated in the liver. Since intestinal absorption of sulfated esters is low and its proportion in urine is greater than bile or serum, only small amounts of sulfate esters have been identified in human serum.[18-21]

Since the hepatic bile acid uptake mechanism normally operates well below saturation, and hepatic extraction efficiency is relatively constant with bile acid loads in the physiological ranges, bile acid levels in healthy people are primarily a reflection of the rate of intestinal input. These serum bile acid levels depend on meal-stimulated gall bladder contraction, malabsorption, or hepatic extraction efficiency. In liver disease the extraction efficiency may be reduced because of either reduced functional hepatocyte mass or shunting of blood past the

hepatocyte, and systemic bile acid levels consequently approach those normally present in the portal circulation.[7,22]

Three methods, such as gas chromatography,[12] enzymatic assay,[23] and radioimmunoassay [24,25] are currently useful for measuring serum bile acids. Radioimmunoassays by commercially available kits can separate individual bile acids and offer the advantages of great sensitivity, precision, and the ability to perform large numbers of assays. In the present study, using radioimmunoassay, although the greatest increase of serum bile acids was found in acute viral hepatitis, mild increase was also noted in chronic active hepatitis and moderate increase in liver cirrhosis and hepatoma groups. These results seem to be caused by quantitative and qualitative change in the hepatic synthesis of bile acid, or destruction of hepatic acini and intrahepatic bile ducts in liver disease.[13] However, since the serum bile acid concentrations overlapped in both acute and chronic liver disease, bile acid detemination was not of help for the differential diagnosis of liver diseases, particularly for deciding whether liver disease is acute or chronic.

It was reported that cholic acid synthesis was a sensitive indicator of hepatocellular damage and its level was inversely correlated with the severity of liver disease.[10] In cirrhosis, synthesis and fractional daily turnover rate of cholic acid are markedly reduced, while synthesis, turnover rate and pool size of chenodeoxycholic acid are affected to a much lesser extent. Although the mechanisms of these changes are not completely understood, an apparent defect in cholic acid synthesis in liver cirrhosis and chronic hepatitis may account for the usual predominence of chenodeoxycholic acid. In cholestatic conditions, preferential sulfation of chenodeoxycholic acid results in enhanced urinary excretion of polar sulfate conjugated bile acids, thus accounting for the predominance of cholic acid in serum.

Some workers have advocated the use of the cholic: chenodeoxycholic acid ratio in differential diagnosis of hepatobiliary diseases.[12,13] Healthy subjects had ratios between 0.6 to 1.0. In cirrhosis the ratio was reduced (0.1 to 0.5), whereas in extrahepatic obstruction it was increased (0.96 to 3.6). Ratios in patients with viral hepatitis and hepatic neoplasms overlapped with those from healthy subjects and cirrhotics. The ratio does not distinguish intrahepatic from extrahepatic cholestasis and these observations are still matters of dispute.

Kaplowitz et al.[3] first suggested that measuring serum bile acids two hours after a meal was the method of choice for detecting hepatobiliary diseases. This theoretical consideration appears that the bulk of bile acids stored in the gall bladder during fasting will return to the liver postprandially through enterohepatic circuit. In liver disease the elevation of postprandial bile acids results from the inability of the liver cell to remove bile acids from portal blood or from portosystemic shunting by which the bile acids bypass hepatocyte. Although some[3,4] failed to show a postprandial increase in serum bile acids in control subjects using gas-liquid chromatography, several studies,[5,9,12,23,26,27] using more sensitive methods, showed a postprandial increase in serum bile acids in control subjects and mild liver disease. Controversy still exists as to the basis for the greater sensitivity of postprandial serum bile acids as compared with fasting levels. The present study showed that the overall increase in postprandial serum bile acids exceeded fasting ones in both control and liver disease groups. The postprandial serum bile acids were increased even when fasting levels were normal. However, we found no statistical difference between the fasting and postprandial levels in each groups and could not demonstrate any consistent advantage of the postprandial bile acids over fasting ones. In fact, increased portal systemic shunting of blood postprandially may contribute, but increased postprandial serum bile acids may be more affected by the qualitative difference in bile acids returning to the liver than physiologic difference between the postprandial and fasting states. Since the type of bile acid presented to the liver is different, the measurement of trihydroxy bile acids that have higher extraction ratio in postprandial state may provide more sensitive detection of impaired hepatic extraction.

In hepatobiliary disease, fasting serum bile acids are frequently elevated even when bilirubin is normal. The greater sensitivity of the serum bile acids may be explained by far greater pool size and flux of bile acids as compared with bilirubin. Thus, a slight defect in excretion of bile acids results in a larger increase in serum bile acids than in bilirubin. The present study showed that fasting serum bile acids were elevated in patients with chronic active hepatitis and liver cirrhosis when their serum bilirubin were within normal limits. As one might expect, measurements of serum bile acids in fasting state are clearly superior to serum bilirubin in the detection of anicteric patients with occult, hepatic disease. Moreover, we showed that fasting serum bile acids had significant correlation with serum enzymes and albumin in each group of liver disease except

hepatoma. In 7 patients with liver cirrhosis with normal transaminases, the fasting serum bile acid concentrations were above normal range, so that they were more sensitive test of disordered hepatobiliary function than conventional liver function tests. This observation was similar to earlier reports.[4-6,12]

The introduction of serum bile acid measurement as a routine laboratory test of liver function depends on the ease with which the estimation can be performed. Analysis by radioimmunoassay appears to be more useful since it is very sensitive and requires only smali amounts of serum. As a sensitive and specific screening test for hepatobiliary disease, the measurement of serum bile acids may have several clinical applications. The two-hour postprandial serum bile acid concentration seems according to the present data to be no more a sensitive indicator of disordered hepatobiliary function than the fasting one, but this possibility deserves further study. Another clinical application of the measurement of serum bile acids appears to be useful in following the progression of viral hepatitis, particularly in the resolving phase when they remain abnormal although other liver functions have returned to normal. And also it can be useful in judging the response to therapy of patients with chronic active hepatitis and in predicting those patients who subsequently relapsed following biochemical and histological resolution.[8,27] In addition, it is suggested that the serum level of bile acid may be used to confirm the hepatic origin of an increased level in serum enzymes such as alkaline phosphatase or aspartate aminotransferase and allow diffential diagnosis of jaundice.[29] Normal levels of serum bile acids in the presence of hyperbilirubinemia appears to occur in congenital hyperbilirubinemia and hemolysis. The validity of these applications requires further study.

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