

Bile acids in serum and bile of patients with cholesterol gallstone

HAN Tian-Quan¹, ZHANG Sheng-Dao¹, TANG Wen-Hao² and JIANG Zhao-Yan¹

Subject headings cholesterol gallstone; bile acids/analysis; deoxycholic acids/analysis; chromatography, gas

Abstract

AIM To analyze serum bile acids and biliary lipids of patients with cholesterol gallstone (CS) and explore the relationship between deoxycholic acid (DCA) and CS disease.

METHODS Analysis of bile acids in serum was done with gas-chromatography in two groups: CS group ($n = 151$) and control group ($n = 256$). Serum bile acids and biliary lipids were also studied in 90 matched samples.

RESULTS The serum DCA was $0.955 \mu\text{mol/L} \pm 0.078 \mu\text{mol/L}$ in CS group, which was more than that of control group ($0.696 \mu\text{mol/L} \pm 0.047 \mu\text{mol/L}$), $P < 0.01$. The ratio of DCA/chenodeoxycholic acids (CDCA) was 1.76 ± 0.30 in CS group, about two times that in control group (0.92 ± 0.14). The mole percent of DCA in bile was positively related to cholesterol saturation index (CSI) ($P < 0.01$) and the mole percent of CDCA in bile negatively to CSI ($P = 0.01$). There was correlation between the mole percent of DCA, CDCA and cholic acid in bile and in serum.

CONCLUSION It is suggested that DCA is lithogenic and the increased amount of DCA or the ratio of DCA/CDCA in serum may be one of the features of cholesterol gallstone patients.

INTRODUCTION

Bile acids may play a role in the formation and dissolution of cholesterol gallstones. Chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) are litholytic bile acids, which can dissolve cholesterol fraction of gallstones and used in prevention. Deoxycholic acid (DCA), however, as one of lithogenic bile acids has attracted great attention^[1]. The purpose of this study was to analyze bile acids in both serum and bile and biliary lipids in cholesterol gallstone patients, and find out the relationship between bile acids and biliary lipids, especially between DCA and biliary lipids.

MATERIALS AND METHODS

Subjects

Two groups were studied The cholesterol gallstone group had 151 patients. Among them 88 had cholesterol gallstones classified by both the observation of the cross section of stones obtained during cholecystectomy and chemical analysis of their components. The other 63 asymptomatic patients had radiolucent gallstones and well-functioning gallbladders shown by oral cholecystography. The control group included 247 healthy individuals by both physical examination and B-ultrasonography and 9 patients without gallstones confirmed at the time of non-biliary surgery. None of the subjects had any evidence of hepatic or renal abnormality, or other diseases such as diabetes, hypertension and coronary heart disease. Both gallbladder bile and venous blood were obtained during elective cholecystectomy. For the nonoperative subjects, only fasting blood was available.

Study protocol

In analysis of serum bile acids, the gallstone group comprised 63 males and 88 females, with an age range of 52.6 ± 11.5 years, and the control group 169 males and 87 females, with an age range of 50.0 ± 10.3 years.

In analysis of matched serum and biliary bile acids, all the subjects had undertaken abdominal operation. Eighty-one with cholecystectomy had cholesterol gallstones and 9 with non-biliary operations had no stone in the gallbladder, including 37 males and 53 females with a mean age of 50.5 ± 11.0 years.

Assay of bile acids

Bile samples were directly hydrolyzed with NaOH.

¹Department of Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China

²Department of Surgery, Affiliated Hospital, Nanjing Railway Medical College, Nanjing 210009, China

Dr. HAN Tian-Quan, male, born on 1950-12-07 in Hangzhou, Zhejiang Province, Graduated from Bengbu Medical College in 1975 and Shanghai Second Medical University in 1989. Associate Professor, Ph. D. of Medicine, tutor of postgraduate students of master degree, specialized in the study of mechanism of gallstone formation, acute pancreatitis and gastrointestinal motility, having 31 papers published. Tel. +86-21-64370045 ext. 6015

*Project Supported by the National Natural Science Foundation of China, No. 850540.

Correspondence to Dr. HAN Tian-Quan, Department of Surgery, Ruijin Hospital, Shanghai Second Medical University, 197 Ruijin Erlu, Shanghai 200025, China

Received 1997-09-20

Two ml blood was extracted through Sep-pak® C₁₈ cartridge and then hydrolyzed with NaOH. Bile acids from both bile and serum were treated as hexafluoroisopropyl ester trifluoroacetyl derivatives and measured quantitatively by a Shimadzu GC-9A gas-chromatograph equipped with electron capture detector^[2]. A glass column packed 1.5% QF-1/Chromsorb W AW DMCS (2.1 m × 3.2 mm ID) was used. The operating procedure was as follows: injection and detector temperature, 300°C; column temperature, 240°C; and nitrogen flow-rate 60 ml/min.

Biliary cholesterol saturation index (CSI)

Biliary cholesterol was measured by enzymatic method and phospholipids by Bartlett's method. The total bile acids (TBA) was summed by DCA, CDCA and cholic acid (CA). CSI was calculated according to the critical table of Carey.

Statistical analysis

The results were expressed as $\bar{x} \pm s_x$. Statistical analysis included both *t* test and correlation test. *P*-values less than 0.05 were considered as significant.

RESULTS

Characteristics of bile acids in serum of gallstone patients

Patients with cholesterol gallstones had significantly higher serum DCA than controls. However, there was no significant difference between the two groups in the other serum bile acids and total bile acids (Table 1). When analyzing the mole percent of serum bile acids (Table 2), we found that DCA was increased in patients with gallstones compared with controls, CDCA was decreased and the ratio of DCA/CDCA increased nearly one-fold.

Correlation between bile acids and CSI

Biliary bile acids were mainly composed of CA, CDCA and DCA with a small fraction of lithocholic acid (LCA). Correlation analysis of 90 samples of gallbladder bile acids showed that DCA and LCA were positively correlated with CSI whereas CDCA was negatively correlated with CSI. No correlation was found between CSI and CA (Table 3). These showed each bile acid had its own characteristics, DCA and LCA were lithogenic whereas CDCA could decrease CSI.

Correlation between biliary and serum bile acids

The concentration of biliary bile acids was much higher than those in serum (Table 4). Significant correlation existed between the mole percent of biliary and serum bile acids, which suggested that serum bile acids concentration might reflect the relative concentration of bile acids in the gallbladder (Table 5).

Table 1 Changes of serum bile acids in the two groups ($\mu\text{mol/L}$, $\bar{x} \pm s_x$)

Bile acids	Group with calculi (<i>n</i> = 151)	Control group (<i>n</i> = 256)
DCA*	0.955 ± 0.078	0.696 ± 0.047
CDCA	2.535 ± 0.304	2.084 ± 0.141
CA	1.202 ± 0.134	1.211 ± 0.147
TBA	4.692 ± 0.432	3.290 ± 0.267

**P* = 0.002.

Table 2 Mole percent of serum bile acids in the two groups (% $\bar{x} \pm s_x$)

Bile acids	Group with calculi (<i>n</i> = 151)	Control group (<i>n</i> = 256)	<i>P</i>
DCA	28.12 ± 1667	23.03 ± 1.04	0.007
CDCA	45.11 ± 2.01	52.40 ± 1.32	0.001
CA	27.51 ± 1.93	24.77 ± 1.09	>0.05
DCA/CDCA	1.76 ± 0.30	0.92 ± 0.14	0.004

Table 3 Correlation between bile acids and biliary CSI (*n* = 90)

Bile acids	Correlation coefficient	<i>P</i>
LCA	0.288	0.006
DCA	0.311	0.003
CDCA	-0.269	0.01
CA	-0.101	>0.05

Table 4 Biliary and serum bile acids (*n* = 90, $\bar{x} \pm s_x$)

Bile acids	Bile (mmol/L)	Serum ($\mu\text{mol/L}$)	Correlation coefficient*
LCA	1.97±0.18		
DCA	24.80±2.12	1.08±0.11	0.12
CDCA	76.93±4.01	2.35±0.43	0.07
CA	40.27±3.08	1.31±0.19	-0.09
TBA	143.97±7.33	4.65±0.62	-0.07

**P*>0.05.

Table 5 Correlation between mole percent of biliary and serum bile acids (*n* = 90, $\bar{x} \pm s_x$)

Bile acids	Bile (mole %)	Serum (mole %)	Correlation coefficient	<i>P</i>
LCA	1.89±0.24			
DCA	17.52±1.06	30.15±2.59	0.445	<0.001
CDCA	54.16±1.12	36.66±2.91	0.434	<0.001
CA	26.43±0.96	33.19±3.04	0.313	0.003

DISCUSSION

Biliary cholesterol supersaturation is a prerequisite for the formation of cholesterol gallstones. A positive correlation was found between serum DCA and CSI in bile of patients with stone^[3]. Therefore it might be possible to predict biliary CSI just by analyzing serum bile acids, but this study was primarily based on inpatients with gallstone. The present study was performed among normal

population, asymptomatic gallstone patients and healthy individuals, rather than inpatients^[3,4]. Among the 407 samples, the mole percent of biliary DCA was found positively correlated with CSI, and serum DCA was increased by about 37% in patients with cholesterol gallstones as compared with control, and the ratio of DCA/CDCA increased nearly one-fold. Hence, there is not only possibility of prediction of CSI in bile, but also characteristic change of DCA in the cholesterol gallstone patients.

Normally, bile acids can be classified into primary bile acids, CA and CDCA, and secondary bile acid, DCA. CA is dehydrogenated to DCA by intestinal bacteria and their secretion is coupled with cholesterol. Bile acid can increase cholesterol secretion and is related to its different hydrophobicity. DCA is more hydrophobic and is followed by greater output of biliary cholesterol than other more hydrophilic bile acids, as CA, CDCA and UDCA. It had been demonstrated by DiDonato *et al*^[5] that biliary DCA increased from 5.3% to 43.9% and bile CSI increased simultaneously from 0.9 to 1.34 after given a dose of 3 mg/kg of DCA orally for three to four weeks. One week after its discontinuance, the biliary CSI returned to the original level. DCA could also promote cholesterol crystal formation. The *in vitro* experiment showed that the nucleation time shortened when the amount of taurodeoxycholic acid increased^[1]. Therefore, DCA had a dual effect on supersaturation and nucleation, which contributed to gallstone formation. It was further confirmed by Berr *et al*^[6] that increase in

hydrophobic bile acids and decrease in its counterparts were the main metabolic disturbance for cholesterol gallstone disease.

Hepatocytes uptake most of the bile acids in the portal vein and then transport them into enterohepatic circulation. The remaining minor portion of bile acids escaped into the systemic circulation are the origin of bile acids in peripheral circulation. They are proportionally related to the composition of bile acid pool^[7]. Whiting *et al* suggested that serum bile acids could reflect biliary bile acids, which was confirmed by the present study. As serum DCA is relevant to the relative concentration of bile DCA, the determination of DCA may be useful for the prediction and guiding preventive treatment of gallstone disease^[8-10].

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