

Biliary Bile Acid Analysis in the Patients with Bile Duct Cancer

Jin Heon Lee, M.D., Yoon Hee Park, M.S.,* Jeong Hun Suh, M.D.,
Jae Bock Chung, M.D., Se Joon Lee, M.D., Joon Pyo Chung, M.D.,
Si Young Song, M.D. and Jin Kyung Kang, M.D.

Department of Internal Medicine and Institute of Gastroenterology,
Yonsei University College of Medicine, Seoul, Korea*

Background/Aims: Bile acids including deoxycholic acid (DCA) have been known as carcinogenic on human colonic mucosa. However, limited data on changes of bile acid composition in the patients with bile duct cancer are available. The aim of this study was to assess the changes in composition of biliary bile acids in patients with bile duct cancer. **Methods:** The bile was collected from 35 patients with bile duct cancer (25 males, 10 females, mean age of 61.4 years) and 23 patients with bile duct stone for control (11 males, 12 females, mean age of 57.7 years) bile acid analysis was then performed by high performance liquid chromatography. **Results:** The total bile acids concentration was not different between two groups. The concentrations of deoxycholic, glycodeoxycholic and taurodeoxycholic acid in the bile of cancer patients were higher than those in the bile of control group. However there was no statistical significance. The fractional ratio of taurocholic acid was higher in cancer patients than in control group ($p=0.008$). **Conclusions:** There was no statistically significant difference in DCA concentration between two groups. However, the fact that the DCA level was higher in the patients with bile duct cancer than in control group suggests the possibility of relationship between bile duct cancer and the changes in bile acid composition. Further study is needed to obtain more reliable results. (**Kor J Gastroenterol 2000;35:103 - 110**)

Key Words: Bile, Bile acid, Bile duct cancer

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Tel: (02) 361-5427, Fax: (02) 393-6884
1996
(HMP-96-M-2-1019)

cholic acid chenodeoxycholic acid
 deoxycholic acid lithocholic acid
 2 chenodeoxycholic acid 7 -epimer
 3 ursodeoxy-
 cholic acid가 . Hamster
 deoxycholic acid

carcinogenesis
 , ursodeoxycholic acid
 .14 deoxycholic
 acid lithocholic acid 2
 .56
 cholic acid deoxycholic acid가
 carcinogenesis 가
 ,78

1.
 1997 1 1999 2
 (ERCP)
 (abdominal CT)
 가
 (PTBD)

(ENBD)
 35 23
 1 3 가 ,
 1 . :
 가 25:10 , 11:12 ,
 61.4 57.7 .
 (Table 1).
 2.
 1)
 8 PTBD ENBD
 (10 cc)
 -70
 2)
 37
 methanol 50
 (vortex) 3,500 rpm 5
 20 µL
 3-hydroxysteroid dehy-
 drogenase (3-HSD), nicotinamide adenine
 dinucleotide (NAD) -
 NADH
 (high pressure liquid chromato-
 graphy, HPLC) ,9,10 HPLC
 JASCO PU-980 intelligent HPLC pump
 , column 4.6 × 125 mm
 Bilepak-II . HPLC Table
 2 . Sigma
 Table 3 .
 cholic acid, taurocholic acid, glycocholic
 acid, deoxycholic acid, taurodeoxycholic acid, glyco-
 deoxycholic acid, lithocholic acid, tauroolithocholic
 acid, glycolithocholic acid, ursodeoxycholic acid,
 tauroursodeoxycholic acid, glyoursodeoxycholic acid,
 chenodeoxycholic acid, taurochenodeoxycholic acid,
 glycochemodeoxycholic acid 15가
 1

Table 1. Clinical Characteristics and Laboratory findings of Bile Duct Cancer Patients and Control Group

	Cancer group (n=35)	Control group (n=23)	p value
Male:Female	25:10	11:12	0.070
Age (years)	61.4 ± 12.9	57.7 ± 13.6	0.311
Range	27 85	29 84	
WBC count (/ μL)	8725.5 ± 2414.8	8823.8 ± 4729.2	0.940
Hemoglobin (g/dL)	12.4 ± 1.8	12.5 ± 1.8	0.919
Platelet count (/ μL)	279666 ± 102480	226461 ± 72749	0.102
Total protein (g/dL)	6.8 ± 0.8	6.9 ± 0.4	0.948
Albumin (g/dL)	3.6 ± 0.5	3.8 ± 0.5	0.210
Total bilirubin (mg/dL)	3.6 ± 3.3	1.9 ± 2.3	0.087
Direct bilirubin (mg/dL)	2.8 ± 2.6	1.1 ± 1.7	0.092
ALP (IU/L)	300.8 ± 209.8	182.2 ± 115.8	0.056
AST (IU/L)	80.9 ± 108.8	79.2 ± 102.3	0.966
ALT (IU/L)	102.2 ± 134.4	117.4 ± 137.3	0.762
-GT (IU/L)	248.3 ± 205.9	198.4 ± 160.9	0.469
P-time (%)	95.7 ± 8.3	93.6 ± 6.8	0.429

Table 2. HPLC Conditions for Determination of Bile Acid

Pump constituents	JASCO PU-980 intelligent HPLC pump			
	JASCO LG-980-02 ternary gradient unit			
	JASCO DG-980-50 3-line degasser			
Injector	JASCO 851-AS intelligent autosampler			
Thermo controller	Column cabinet CS-3000C (Chromato Science Co., LTD)			
Detector	JASCO FP-210 fluorescence detector (Ex 345 nm, Em 470 nm)			
Integrator	JASCO 807-IT integrator			
Column	Bilepak-II (4.6 × 125 mm)			
Mobile Phase	Ⓢ CH ₃ CN : CH ₃ OH : 30 mM ammonium acetate + 30 : 30 : 40			
	Ⓢ CH ₃ CN : CH ₃ OH : 30 mM ammonium acetate + 20 : 20 : 60			
	Ⓢ (0%)	32 mm	Ⓢ (100%)	28 min
	Ⓢ (100%)	Linear	Ⓢ (0%)	Ⓢ (0%)
Temperature	20			
Flow rate	1.0 ml/min			
Injection volume	20 μl			
Immobilized enzyme column	Enzymepak-HSD (4.6 × 35 mm)			
Reagent	(0.3 mM NAD + 10 mM KH ₂ PO ₄) + 1 mM EDTA-2Na + 0.05% 2-mercaptoethanol/L, pH 7.78-7.8			
Reagent flow rate	1.0 ml/min			

Table 3. Amounts and Concentrations of Each Bile Acid in the Standard Solution (500 mL)

Component	Amount (mg)	Concentration (µg/ml)
CA (cholic acid)	11.01	22.02
DCA (deoxycholic acid)	10.07	20.14
CDCA (chenodeoxycholic acid)	11.44	22.88
UDCA (ursodeoxycholic acid)	11.17	22.34
LCA (lithocholic acid)	12.02	24.04
GCA (glycocholic acid)	11.21	22.42
GDCA (glycodeoxycholic acid)	11.96	23.92
GCDCA (glycochenodeoxycholic acid)	11.20	22.40
GLCA (glycolithocholic acid)	11.23	22.46
GUDCA (glycoursodeoxycholic acid)	12.75	22.50
TCA (taurocholic acid)	11.32	22.64
TDCA (taurodeoxycholic acid)	11.21	22.42
TCDCA (taurochenodeoxycholic acid)	11.69	23.38
TLCA (taurolihocholic acid)	10.37	20.74
TUDCA (tauroursodeoxycholic acid)	10.75	21.50

GDCA TDCA
 220.1 115.2 µg/mL 44.1 27.7 µg/
 mL
 (Table 4).

2.

DCA, GDCA TDCA
 가
 , TCA
 18.6% 10.1%
 (p=0.008).

UDCA 0.042%
 0.027%
 (p=0.634)(Table 5).

3. 1 2

CA CDCA 1 DCA LCA
 2 1.74
 ±6.30% 0.93 ±1.74%
 (p=0.480).

1.
 15 6165.2 ±9156.7 µg/mL
 6689.6 ±5593.1 µg/mL 가
 (p=0.788), DCA
 1.4 µg/mL 0.3 µg/mL ,

4)
 ± ,
 chi-square test in
 dependent sample t-test , p 0.05
 .

Table 4. Concentrations of Each Bile Acid in Both Group

Conc. of bile acid (µg/mL, Mean ± SD)	Cancer group (n=35)	Control group (n=23)	p value
CA (cholic acid)	12.0 ± 39.1	36.1 ± 122.1	0.279
DCA (deoxycholic acid)	1.4 ± 5.5	0.3 ± 1.0	0.251
CDCA (chenodeoxycholic acid)	4.7 ± 16.6	25.8 ± 96.3	0.208
UDCA (ursodeoxycholic acid)	7.8 ± 45.6	2.8 ± 7.7	0.528
LCA (lithocholic acid)	0.1 ± 0.7	0 ± 0	0.312
GCA (glycocholic acid)	1516.0 ± 2060.3	2679.9 ± 2633.8	0.081
GDCA (glycodeoxycholic acid)	220.1 ± 1266.1	44.1 ± 69.8	0.418
GCDCA (glycochenodeoxycholic acid)	1698.8 ± 2824.0	1902.1 ± 1695.0	0.733
GLCA (glycolithocholic acid)	35.0 ± 123.2	4.4 ± 20.0	0.243
GUDCA (glycoursodeoxycholic acid)	305.6 ± 584.6	246.5 ± 486.0	0.678
TCA (taurocholic acid)	829.3 ± 1156.5	773.1 ± 907.1	0.837
TDCA (taurodeoxycholic acid)	115.2 ± 425.2	27.7 ± 49.4	0.331
TCDCa (taurochenodeoxycholic acid)	1335.5 ± 2959.7	912.4 ± 1354.5	0.465
TLCA (tauroolithocholic acid)	6.4 ± 22.1	0.9 ± 4.2	0.245
TUDCA (tauroursodeoxycholic acid)	77.1 ± 201.9	33.4 ± 68.0	0.243

Table 5. Fractional Ratio of Each Bile Acid in Both Group

Fractional ratio of each bile acid (%)	Cancer group (n=35)	Control group (n=23)	p value
CA (cholic acid)	0.2 ± 0.6	0.9 ± 2.3	0.085
DCA (deoxycholic acid)	0.01 ± 0.02	0.01 ± 0.02	0.729
CDCA (chenodeoxycholic acid)	0.1 ± 0.3	0.5 ± 1.6	0.123
UDCA (ursodeoxycholic acid)	0.03 ± 0.12	0.04 ± 0.11	0.634
LCA (lithocholic acid)	0.01 ± 0.01	0.00 ± 0.00	0.309
GCA (glycocholic acid)	34.0 ± 15.8	42.6 ± 17.1	0.061
GDCA (glycodeoxycholic acid)	0.7 ± 3.2	0.5 ± 0.8	0.638
GCDCA (glycochenodeoxycholic acid)	23.0 ± 10.8	28.4 ± 8.8	0.045
GLCA (glycolithocholic acid)	0.11 ± 0.38	0.05 ± 0.23	0.500
GUDCA (glycoursodeoxycholic acid)	4.9 ± 7.1	4.2 ± 7.0	0.685
TCA (taurocholic acid)	18.6 ± 14.3	10.1 ± 5.4	0.008
TDCA (taurodeoxycholic acid)	0.5 ± 1.2	0.3 ± 0.4	0.301
TCDCa (taurochenodeoxycholic acid)	16.5 ± 12.7	11.9 ± 10.6	0.143
TLCA (tauroolithocholic acid)	0.02 ± 0.68	0.01 ± 0.05	0.561
TUDCA (tauroursodeoxycholic acid)	1.3 ± 2.2	0.7 ± 1.4	0.248

4. stage IV- A (p=0.560),
 1 . 가
 11 24 20 가 stage IV-A
 3 , : 가 13:7
 7432.8 ± 2673.3 µg/mL 59.6 ± 13.0 ()
 32 6046.3 ± 9556.5 µg/mL p=0.258, p=0.641). 6556.1

± 8941.0 µg/mL
DCA TDCA 2.5 ± 7.1
83.9 ± 236.0 µg/mL

(p=0.154, p=0.271).
GDCA 8.9 ± 19.5
µg/mL (p=0.035).

bile acid 가
.13

carcinogenesis
deoxycholic acid 2

chemical

chemical carcinogen

cocarcinogenicity가

가

cholesterol 7 -hydroxylase sterol
27-hydroxylase

.2

.11 cholic acid, chenodeoxycholic
acid, deoxycholic acid lithocholic acid
taurine glycine

가

-OH (amphi-
philic) , -OH 가

deoxycholic acid, glycodeoxycholic acid
taurodeoxycholic acid

-COOH 가 deoxycholic acid

, deoxycholic acid
가 taurocholic acid

가
.9,12

carcinogenesis
.1,2,7,8 urso-

ursodeoxycholic acid

deoxycholic acid

(high pressure liquid chromatography, HPLC)

.34

deoxycholic acid lithocholic
acid 2

deoxycholic acid 가

deoxycholic acid 2

가 가
가

.6

.12

deoxycholic acid, lithocholic acid unconjugated

가

가

1 2

가

10

3.5

.14

1.9 . Stage

IV-A deoxycholic acid :

가 가

가 35 , 23

deoxycholic acid carcinogenesis

(HPLC) 15가

가 , :

6165.2 µg/mL 6689.6 µg/mL

가 (p=0.788), DCA

1.4 µg/mL 0.3 µg/mL ,

가 GDCA TDCA

220.1 115.2 µg/mL 44.1 27.7 µg/mL

가 ,

DCA, GDCA TDCA

가

, TCA

18.6% 10.1%

(p=0.008). CA CDCA 1

DCA LCA 2

1.74% 0.93%

(p=0.480). Stage

IV-A 20 DCA

TDCA 2.5 83.9 µg/mL

(

p=0.154, p=0.271), GDCA 8.9 µg/mL

(p=0.035). :

: deoxy-

cholic acid

carcinogenesis

2 가

가 가

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 9. , , , , .
deoxycholate immunoglobulin G가
1996;28:845-852.
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