ISSN 0025-7680

ORIGINAL ARTICLE

MEDICINA (Buenos Aires) 1999; 59: 269-273

FECAL BILE ACID EXCRETION PROFILE IN GALLSTONE PATIENTS

ARNALDO MAMIANETTI[1], DELIA GARRIDO[3], CLYDE NORA CARDUCCI[2], MARIA CRISTINA VESCINA[2]

[1] *Departamento de Medicina Interna, Hospital Aeronáutico Central;* [2] *Departamento de Química Analítica y Fisicoquímica y* [3] *Departamento de Físico-Matemática, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires*

Abstract Epidemiological studies have shown a positive association between cholesterol gallstones and colonic cancer. These two diseases may be somehow related with bile acids metabolic alterations. The aim of this study was to evaluate the profiles of fecal bile acid in gallstone patients, in order to estimate the quality and amount of fecal bile acids. A fecal bile acid profile of ten gallstone patients and ten controls was compared using high performance liquid chromatography. Total fecal bile acid excretion was significantly increased in gallstone patients compared with controls (692.7 mg/day (302.5-846.2) vs 165.7 mg/day (138.7-221.3), $p < 0.01$) as was the excretion of secondary free bile acids 562.9 mg/day (253.3-704.9) vs 99.9 mg/day (88.9-154.2), $p < 0.01$). Lithocholic and glycodeoxycholic acid percentages have also been found to show differences with controls of 55.4 (47.4-73.9) vs 24.6 (22.1-38.4) ($p< 0.01$) and 29.4 (3.3-41.7) vs 2.8 (1.0-3.8) ($p < 0.03$), respectively but deoxycholic acid has not shown differences between the two groups. Moreover, the percentage of ursodeoxycholic acid diminished significantly in gallstone patients (1.5 (1.0-2.8) vs 8.6 (6.0-10.39) ($p < 0.001$), and the decrease of chenodeoxycholic acid was also significant (20.0 (11.4-23.6) vs 8.9 (3.1-10.9) ($p < 0.03$) along with a rise in the ratios lithocholic/deoxycholic acids (1.8 (1.4-6.4) vs 0.9 (0.6-1.6) ($p < 0.05$) and glycine/taurine of deoxycholic acid (7.3 (4.1-46.6) vs 0.2 (0.1-0.5) ($p < 0.01$). In conclusion, we have observed a significant increase of total and secondary fecal bile acid excretion as well as a rise of LCA and GDCA percentages and a rise in the ratios of LCA/DCA and glycinet/taurine of DCA.

Resumen ***Perfil de excreción de ácidos biliares fecales en pacientes con cálculos vesiculares.*** Estudios epidemiológicos han mostrado una asociación entre los cálculos vesiculares de colesterol y cáncer colónico. Estas dos enfermedades podrían estar relacionadas con una alteración metabólica de los ácidos biliares. El perfil de los ácidos biliares fecales de 10 pacientes portadores de cálculos vesiculares asintomáticos fueron comparados con 10 sujetos controles usando cromatografía líquida de alta resolución. La excreción total de los ácidos biliares fue significativamente más elevada en los pacientes litiásicos que en los controles (692.7 mg/día (302.5-846.2) vs 165.7 mg/día (138.7-221.3), $p < 0.01$) así como la excreción de los ácidos biliares libres secundarios (562.9 mg/día (253.3-704.9) vs 99.9 mg/día (88.9-154.2), $p < 0.01$). Los porcentajes de los ácidos litocólico y glicodesoxicólico también mostraron una diferencia significativa respecto de los controles de 55.4 (47.4-73.9) vs 24.6 (22.1-38.4) ($p < 0.01$) y 29.4 (3.3-41.7) vs 2.8 (1.0-3.8) ($p< 0.03$), respectivamente, pero el ácido desoxicólico no mostró diferencias entre los dos grupos. Además se halló que el porcentaje del ácido ursodesoxicólico disminuyó significativamente en los pacientes litiásicos (1.5 (1.0-2.8) vs 8.6 (6.0-10.39) $p < 0.001$), también el descenso del ácido quenodesoxicólico resultó significativo en el mismo grupo (20.0 (11.4-23.6) vs 8.9 (3.1-10.9) $p < 0.03$), y fue observado un aumento significativo de las relaciones ácido litocólico/desoxicólico (1.8 (1.4-6.4) vs 0.9 (0.6-1.6) $p < 0.05$) y ácidos glicodesoxicólico/taurodesoxicólico (7.3 (4.1-46.6) vs 0.2 (0.1-0.5) $p < 0.01$). En conclusión, se observó en los pacientes litiásicos un aumento significativo en la excreción de ácidos biliares fecales totales y secundarios así como un aumento de los porcentajes de LCA y GDCA y también en las relaciones LCA/DCA y glico/tauro derivados del DCA.

Key words: fecal bile acids, gallstones, HPLC

A positive association between colonic cancer and gallstones was demonstrated in post-mortem and clinical studies[1-3], although some reports found no obvious association[4, 5]. A possible explanation for the association between colonic cancer and gallstones is the existence of risk factors common to both diseases[6, 7]. It was suggested that dietary factors such as high intake of animal fat, animal protein and low fibre intake play an important role in determining the relative risk for the development of gallstones and colonic cancer[8, 9], probably influenced by genetically determined susceptibility.

People eating a high fat[10] and beef diet[11] induce colonic bacteria changes which produce larger amounts of 7-α-cholesterol dehydroxylase, the enzyme presumably involved in the conversion of primary bile acids, cholic

Received: 7-X-1998 Accepted: 17-III-1999

Postal address: Dr. Arnaldo Mamianetti, Hospital Aeronáutico Central, Ventura de la Vega 3967, 1437 Buenos Aires, Argentina
Fax: 54-11-4912-7582. E-mail: mvescina@ffyb.uba.ar

acid (CA) and chenodeoxycholic acid (CDCA) to secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA). In this respect, Moorehead and Mc Kelvey[12] believe that both colonic cancer and gallstone disease may be related to bile acid abnormalities, thus giving a biological expalantion for this association.

Hill et al.[13] were the first to show that fecal bile acid excretion was increased in colorectal cancer patients. Later studies showed that this increase of fecal bile acids was produced by secondary bile acids[14] in colon cancer patients, although these findings were not ratified by other authors[15]. This discrepancy may be due to the fact that experimental designs and methodology of analysis were different[12].

As little is known about fecal bile acid excretion in gallstone patients, the aim of this study was to evaluate the profiles of fecal bile acid in gallstone patients, in order to estimate the quality and amount of fecal bile acids.

Patients and Methods

Ten asymptomatic gallstone patients who had a functioning gallbladder, documented by visualization and by contraction on oral cholecystography or ultrasonography were selected. Except for one subject, all patients had radiolucent stones, the number of which ranged from one to multiple and the sizes did not exceed 2 cm in diameter. They were studied in comparison to ten non-gallstone patients (controls) documented by ultrasonography. Both groups were of latin origin and living in Buenos Aires. Their ages, sexes and weights are shown in Table 1. None of the patients had any gastrointestinal operations other than appendicectomy, nor showed any evidence of hepatic or digestive organic diseases, nor received any antibiotics three months prior to the study. Individuals more than 40% in excess over ideal body weight were excluded. Fifteen days before the collection of feces, all individuals were on 30 kcal/kg body weight/day diet of carbohydrates (50%), fats (30%) and proteins (20%), with strict instructions to follow the diet at home. They had to fill in a form stating what they ate and show it to the research group every 3 (three) days. The subjects who did not do so were excluded from the experiment.

The protocol had been approved by the Ethics Committee of this Hospital. All participants gave their written informed consent before the study.

Analytical procedure: Feces were home collected for 3 consecutive days, frozen immediately and stored at -20°C until they were analyzed. Data on fecal mass and stool frequency are given in Table 2. The stools were processed as previously detailed[16]. Briefly, they were pooled and homogenized with cold distilled water in a stepwise manner. Fecal bile acids were extracted from 5 mL of fecal homogenate by sequential alcoholic refluxes. After purification, fecal bile acids were separated in free and conjugated derivatives by solid phase columns Bond Elut (Analytichem International, Harbor, City, CA, USA) silica cartridges by selective eluents. Individual bile acids in each fraction were then analyzed by reversed phase-high performance liquid chromatography. For the chromatographic analysis of conjugated bile acids the system previously proposed by the work group was taken as the starting point and all the methodology was properly validated[17].

Adding up free and conjugated bile acids: CA, CDCA, LCA, DCA and ursodeoxycholic acid (UDCA) represent total fecal bile acids. The addition of free bile acids (LCA, DCA and UDCA) constitute free secondary bile acids, and the sum of CA and CDCA primary bile acids.

Statistical analysis: The data were analysed applying the Kruskall-Wallis' method one way analysis of variance by ranks[18]. Results are expressed as medians (25%ile-75%ile), and $p < 0.05$ was considered significant.

TABLE 1.– *Clinical data of control subjects and gallstone patients*

	Control subjects	Gallstone patients
Number of patients	10	10
Sex ratio (female/males)	5/5	6/4
Age (years)	43.7 (35-48)	54.6 (40.69)
Body weight (kg)	69.4 (56-82)	67.7 (54-90)

TABLE 2.– *Stool mass and stool frequency*

	Control subjects n = 10	Gallstone patients n = 10
Fecal wet weight (g/day)	96.0 ± 14.3	102.8 ± 16.7
Fecal dry weight (g/day)	24.5 ± 5.7	23.5 ± 4.0
Water content (%)	76.8 ± 1.9	77.1 ± 1.0
Stool frequency (per day)	1.0 ± 0.1	1.2 ± 0.1

Results are expressed as means ± SEM

Results

The patients did not present any significant difference in stool mass and stool frequency (Table 2). Most bile acids were in their free form and only a small proportion was conjugated with glycine and taurine (Table 3).

Total and free fecal bile acid daily excretion (mg/day) showed a significant increase ($p < 0.01$) (Table 3) and an increase of secondary free fecal bile acids ($p < 0.01$) (Table 3), was also seen in gallstone patients compared with controls.

On studying the excretion pattern of free fecal bile acids expressed in percentages, a few significant alterations in gallstone patients were observed, such as a rise of LCA ($p < 0.01$) (Table 4) and a significant fall of UDCA ($p < 0.001$) and CDCA percentages ($p < 0.03$) (Table 4) and DCA percentages remained the same (Table 4). The ratio secondary/primary free fecal bile acids showed a difference in gallstone patients ($p < 0.02$) (6.2 (4.0-8.3) compared with the control group (2.4 (1.6-5.1).

TABLE 3.– *Values of total, free and conjugated fecal bile acids in control subjects and gallstone patients*

	Control subjects n = 10	Gallstone patients n = 10
Total FBA (mg/day)	165.7 (138.7-221-3)	692.7 (302.5-846.2)*
Free FBA (mg/day)	160.8 (125.3-217.3)	654.1 (290.4-830.2)*
(%)	97.4 (95.2-98.1)	97.5 (94.0-98.2)
primary (mg/day)	59.4 (23.8-65.2)	79.0 (53.1-132.5)
secondary (mg/day)	99.9 (88.9-154.2)	562.9 (253.3-704.9)*
Conjugated FBA (mg/day)	4.4 (3.8-7.7)	16.4 (15.3-25.0)***
%	2.6 (1.9-4.9)	2.5 (1.8-6.0)
primary (mg/day)	1.5 (1.1-4.6)	4.8 (2.5-13.8)
secondary (mg/day)	2.5 (1.5-3.5)	11.6 (4.7-12.9)**

The results are expressed as median (25%ile-75%ile)
** p < 0.01; ** p < 0.03, *** p < 0.5*

TABLE 4.– *Free fecal bile acid profile in control subjects and gallstone patients*

Free bile acid	Control subjects n = 10 %	Gallstone patients n = 10 %
UDCA	8.6 (6.0-10.3)	1.5 (1.0-2.8)*
CA	6.5 (3.9-15.6)	2.8 (2.0-7.1)
CDCA	20.0 (11.4-23.6)	8.9 (3.1-10.9)***
DCA	27.3 (23.8-35.7)	26.7 (12.2-36.4)
LCA	24.6 (22.1-38.4)	55.4 (47.4-73.9)**

Ursodeoxycholic acid (UDCA), cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA).
The addition of free fecal bile acids is 100%
The results are expressed as median (25%ile-75%ile)
** p < 0.001; ** p < 0.01, *** p < 0.03*

TABLE 5.– *Conjugated fecal bile acid profile in control subjects and gallstone*

Conjugated fecal bile acid	Control subjects n = 10 %	Gallstone patients n = 10 %
TUDCA	0.0 (0.0-1.8)	0.0 (0.0-0.9)
TCA	0.7 (0.0-3.3)	2.1 (0.8-4.3)
TCDCA	19.7 (9.1-28.8)	15.8 (5.2-19.4)
TDCA	8.3 (4.4-23.2)	2.2 (1.0-4.7)
TLCA	2.1 (0.2-10.0)	1.7 (0.6-2.9)
GUDCA	0.0 (0.0-1.8)	2.2 (0.1-5.4)
GCA	14.0 (6.8-17.4)	2.6 (0.2-9.5)
GCDCA	9.9 (3.6-18.5)	7.3 (4.0-10.2)
GDCA	2.8 (1.0-3.8)	29.4 (3.3-41.7)*
GLCA	10.9 (3.1-20.1)	13.4 (5.0-19.9)

Tauroursodeoxycholic acid (TUDCA), taurocholic acid (TCA), taurochenodoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), taurolitocholic acid (TLCA), glycoursodeoxycholic acid (GUDCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA) and glycolitocholic acid (GLCA).
The addition of conjugated fecal bile acids (FBA) is 100%
The results are expressed as median (25%ile-75%ile)
** p < 0.03*

The ratios: a) LCA/CDCA showed a significant difference: 7.6 (5.6-19.8) in gallstone patients, 1.2 (1.0-3.6) in controls ($p < 0.01$); b) LCA/UDCA: 32.4 (24.7-38.7) in gallstone patients and 2.5 (1.9-5.8) in controls ($p < 0.001$); c) LCDA/DCA: 1.8 (1.4-6.4) in gallstone patients and 0.9 (0.6-1.6) in controls ($p < 0.05$).

The ratio glycine/taurine in both groups was similar: 2.8 (1.5-3.1) in gallstone patients and 1.0 (0.5-3.5) in the control group. However, on evaluating the ratio glycine/taurine of DCA, it was significantly increased in gallstone patients: 7.3 (4.1-46.6) compared with controls: 0.2 (0.1-0.5) ($p < 0.01$) since glycodeoxycholic acid (GDCA) percentages were found to be increased in gallstone patients ($p < 0.03$) (Table 5).

Discussion

In 1979, Podesta et al.[19] determined fecal bile acids in five gallstone patients and found no difference with healthy controls. These results cannot be compared with our data because 1) the number of days for stool collection was not reported, and Setchell et al.[20] in 1987 pointed out that a 3 to 5 consecutive-day stool collection minimized fecal

bile acids intrasubjetc variability; 2) the sample treatment used in that work produced artifacts[21] and the glycine and taurine conjugation profile could not be analyzed since the sample required previous hydro-lysis for bile acid determination. However, conjugated bile acid profile may be of relevant significance[22].

In the current study we found that free and conjugated bile acid percentages were similar in the two groups. The results were similar to those shown by Setchell et al.[20] in healthy subjects.

The increase of total fecal bile acid excretion we observed in gallstone patients was mainly due to an increase of free bile acids, specially free secondary bile acids, soo that an increase of the ratio secondary/primary bile acids was produced. Non-obese cholesterol gallstone patients increased enterohepatic recycling frequency of bile acids[23], and a slower intestinal transit was shown in gallstone women[24]. Therefore, the enzymatic degradation of primary bile acids to secondary bile acids by intestinal microflora would be increased in our study.

We found an increased proportion of LCA and a reduction of UDCA and CDCA. Also, an increase of the ratios LCA/UDCA and LCA/CDCA was observed.

Those bile acid modifications might be explained taking into account that LCA is formed either by 7-β-dehydroxylation of UDCA or via 7-α-dehydroxylation of CDCA[25, 26].

Since we found a higher LCA/DCA ratio in the stools of gallstone patients, this ratio might have biological relevance[27].

In the fecal conjugated secondary bile acid fraction in gallstone patients under study showed a significant increase of GDCA percentage as well as of the ratio glycine/taurine of DCA. These results could be explained because a possible change in the intestinal flora of our gallstone patients could produce preferential deconjugation[28].

Glycine and taurine conjugation of bile acids in humans modify their biological activity and despite the fact that the amount of conjugated forms in feces are reduced[29], glycine conjugation increases bile acid lipophilicity which might be directly related to its cytolytic[30] and comutagenic effects[31].

In conclusion, we have observed a significant increase of total and secondary fecal bile acid excretion as well as a rise of LCA and GDCA percentages and a rise in the ratios of LCA/DCA and glycine/taurine of DCA.

Acknowledgements: This work was supported in party by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas). We thank, Pharmacists S. Lucangioli, V. Rodriguez and Biochemist N. Vizioli, for their collaboration in the experiments. We are also grateful to Dr. A. Orden for his assistance in the selection of patients and to Ms. G. Pezzo for the English correction.

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La unidad de América Hispánica, o Ibérica, ¿dónde está? ¿Cómo se manifiesta? Y, sin embargo, es cierto que hay un alma americana alentadora, impulsadora de ese mundo familiar nuestro... Hay un misterioso corazón único. Hay una sensibilidad única.

Manuel Aznar

Identidad, Integración y Creación cultural en América Latina. El desafío del Mercosur.
Gregorio Recondo. Buenos Aires: Editorial de Belgrano/Unesco, 1997, p 238