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Biliary bile acids in cholelithiasis and colon cancer

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SUMMARY **The role of biliary deoxycholate as an endogenous colon carcinogen and the possible association between cholelithiasis and/or cholecystectomy and the subsequent development of large bowel cancer is unclear. This paper describes biliary bile acids analysis performed on 13 patients undergoing cholecystectomy for gall stones, 10 patients undergoing colonic resection for colon cancer, and eight control patients. For all 31 patients the total bile acids concentration was highly variable (8·3 mg/ml–106·5 mg/ml). The median ratio of primary to secondary bile acids was 2·7:1. The biliary bile acid ratios were similar in both control patients (3·7:1) and those with colon cancer (3·1:1), whereas patients with gall stones had significantly higher secondary bile acid levels in their biliary bile (ratio 1·9:1, p=<0·05). This result indicates that raised biliary deoxycholate concentrations are not present in patients with colon cancer and are therefore unlikely to be a major predisposing factor in the aetiology of this disease. It is unlikely that cholelithiasis and/or cholecystectomy predispose to the subsequent development of colon tumours.**

Many studies have reported a positive association between the incidence of cholelithiasis or cholecystectomy and colon cancer.[1–16] In some reports the significant association was reported to be sex-specific (women) and/or site-specific (right sided colon tumours) and/or geographically specific (Western diet countries). As a result, the theory that either cholelithiasis or cholecystectomy does indeed predispose to colon cancer has become almost an accepted dogma. This theory has evolved from the studies of Hill and coworkers[17–20] who showed a higher incidence of colon cancer in populations containing relatively more strains of gut anaerobes able to degrade the primary bile acid cholic acid to the secondary bile acid deoxycholic acid, and the close resemblance between dexycholic acid and known carcinogens such as methylcholanthrene. Hill[21] has proposed that excessive exposure of the colonic mucosa to degraded bile salts, especially deoxycholate (such as occurs in gall bladder disease[22 23]) is a causative factor in the development of colon cancer. Because the type of bacterial flora present in the gut is intimately related to the diet, the now familiar, but so far unproven, association of colon cancer with diet could also be linked in this hypothesis.[21]

Contrastingly, in 1973, Doouss and Castleden[24] in a study of 1257 autopsies were unable to find a predicted association between the incidences of gall stones and carcinoma of the large bowel. Subsequently, Hoare[25] and Castleden *et al*[26] reported on the lack of association between cholecystectomy and carcinoma of the colon in retrospective case-control studies. More recently, further reports have appeared questioning the postulated relationship between cholelithiasis and/or cholecystectomy and the subsequent development of colorectal cancer.[27–36] Most of these have been retrospective case control studies. We have now attempted to examine the question in another way, by comparing the biliary bile acids distribution in patients with colon cancer or gall stones and a control group.

Methods

SUBJECTS

Patients in the study groups were undergoing

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cholecystectomy for gall stones (n=13), or colonic resection for colon cancer (n=10). Patients in the control group were undergoing highly selective vagotomy or repair of hiatus hernia (n=8). Of the 13 patients undergoing cholecystectomy, four had functioning gall bladders on oral cholecystography. None of the colon cancer or control patients had gall stones or previous cholecystectomy. Their gall bladders appeared normal at operation and did not contain stones on palpation. They were all therefore presumed to have normal functioning gall bladders, although not all had oral cholecystograms. Informed consent was obtained from all patients, and prior approval of the relevant hospital Ethics in Human Experimentation Committees were obtained.

BILE ACIDS ANALYSIS

Bile was obtained by fine needle aspiration from the gall bladder at operation and processed fresh. The bile specimen (0·1 to 0·2 ml) was deproteinised by drop wise addition to a centrifuge tube containing 0·1 ml [^{14}C]-deoxycholate (2 μCi/ml) and 5 ml methanol. Protein was precipitated by heating for five minutes at 37°C with shaking, then centrifuged at 2000×*g* for five minutes and washed twice with 2 ml methanol. The combined supernatants were made to 70% with water and neutral steroids were removed by three 5 ml petroleum ether extractions. The remaining water methanol mixture containing bile acids conjugates, was evaporated to dryness at 40°C and the residue dissolved in 4 ml 1·25 M aqueous KOH and saponified at 15 psi for three hours. After acidifying with 5 ml 20% NaCl and 5 ml concentrated HCl, bile acids were extracted in diethyl ether (4×5 ml volumes), and adjusted to 25 ml volume. Thereafter, the ether was evaporated, the residue redissolved in methanol, and the bile acids converted to their methyl esters with ethereal diazomethane as described by Klaassen.[37] These were further purified by preparative TLC.[38] The region containing the bile acids methyl esters was aspirated from the plate and eluted with 20 ml ethyl acetate. This extract was evaporated to dryness, redissolved in 5 ml methanol and aliquots taken for scintillation counting to calculate recoveries in the extraction procedure.

Dehydrochlate methyl ester (100 ug) was added as an internal standard to duplicate 1 ml aliquots of the methanol solution which were then evaporated to dryness and redissolved in chloroform (0·5 ml) for conversion of the bile acid methyl esters to their trifluoroacetyl derivatives[37] before gas chromatography.

Dual channel FID analysis was carried out on a Varian 3700 gas-liquid chromatograph equipped with a Hewlett-Packard 3380A recording integrator. Columns were 1·82 m by 2·5 mm glass containing 3% SP-2401 on 100/120 Superlcoport. Column temperature was held at 240°C for one minute after injection and then programmed at 1°C/minute to 256°C where it was maintained for three minutes. Injector and detector temperatures were 305°C and carrier gas was N_2 at a flow rate of 30 ml/min.

A mixture of bile acid standards was processed with each batch of samples. In order to quantify the patients' bile acid concentrations, an internal standard, 7-ketolithocholic acid, was added to every specimen and standards mixture, and a set of standards was processed simultaneously through the extraction procedure. Another set of standards was derivatised at the end of the procedure. By reference to the area ratio of the internal standard peak, the recoveries of the individual extracted standard bile acids was calculated, and these recovery factors were then applied to the calculation for the recovery of the individual sample bile acids. Reproducibility of the area measurements between replicate samples was such that we were able to quantify amounts of bile acid which represented as little as 1% of the total standards mixture.

Results

In all, 13 patients with gall stones, 10 patients with cancer of the colon and eight control patients were studied. The biliary bile acid distributions obtained from these groups of patients are shown in Tables 1, 2, and 3 respectively. As will be seen, there was considerable variation in the concentration of total bile acids in mg/ml of gall bladder bile in all patients and in all groups of patients. There was no statistically significant variation between any two groups.

The percentage concentration of the individual bile acids in the gall bladder bile are shown in Tables 1, 2, and 3 as well as the proportions of primary and secondary bile acids. When all 31 patients are considered, the mean percentage of secondary bile acids was 27·7% of the total concentration of bile acids in the gall bladder bile. The median value was 27%. The dotted lines in the column listing the percentages of secondary bile acids in Tables 1, 2, and 3, separates those patients in each group situated on, above or below the median value.

Because the ratios of primary to secondary bile acids were not normally distributed, non-parametric statistical methodology (the Wilcoxon's rank-sum test), was used to compare the patients in each of the study groups. The patients with gall stones had significantly higher concentrations of secondary bile acids in their biliary bile than the patients in the other two groups ($p<0·05$). There was no significant difference between control and colon cancer groups. These values are shown in Table 4.

Table 1 *Gall stone patients (n=13)*

Patient number	*Sex*	*Total bile acids mg/ml*	*DC %*	*C %*	*LC %*	*CDC %*	*UDC %*	*Primary bile acids %*	*Secondary bile acids %*	
34	M	11·33	6	63	1	29	2	92	8	
7	M	10·55	9	40	1	48	3	88	12	
8	M	16·07	13	49	1	35	2	84	16	
28	M	74·41	16	37	3	38	6	75	25	
26	F	29·83	20	32	2	41	5	73	27	
25	M	35·14	25	28	5	37	5	67	33	median 27%
21	F	50·14	26	24	3	41	6	65	35	
1	M	41·67	28	38	2	29	4	65	35	
22	M	75·37	30	32	4	32	3	64	36	
13	F	17·95	38	25	7	29	1	54	46	
30	F	19·03	49	16	3	30	3	46	54	
17	F	23·82	49	19	4	26	2	45	55	
15	M	54·56	56	15	9	18	2	33	67	
Mean %		35·38	28·1	32·2	3·5	33·3	3·4	65·5	34·5	
(SD)		(22·46)	(16·0)	(13·6)	(2·4)	(7·8)	(1·7)	(17·5)	(17·5)	

DC – deoxycholic acid; C – cholic acid; LC – lithocholic acid; CDC – chenodeoxycholic acid; UDC – ursodeoxycholic acid.

Discussion

In this study we have shown that patients with colon cancer have normal concentrations of deoxycholate in their biliary bile, whilst patients with gall stones (before cholecystectomy) have significantly higher biliary deoxycholate concentrations. This is in accord with work by van der Linden *et al*[29] who studied 18 patients with functioning gall bladders both before, and two to three months after, cholecystectomy. They did not find any change in the concentration of secondary bile acids in these patients but confirmed, as we have done, that patients with gall stones tend to have higher concentrations of secondary bile acids in their bile even before cholecystectomy.

Moorehead *et al*[39] recently reported increased chenodeoxycholic acid and reciprocally reduced cholic acid in the duodenal bile of 14 carcinoma patients compared with age and sex matched controls. This compliments the findings of Owen *et al*[40] that the ratio of faecal lithocholic acid to deoxycholic acid is increased in carcinoma patients and in groups with a relatively high risk of developing colon carcinoma.

In the present study there were wide variations between individuals in each group in both total and individual bile acid concentrations in biliary bile, but in contrast with the findings of Moorehead *et al*,[39] there were no differences in the mean percentages of individual bile acids when colon cancer patients were compared with controls. This same observation was made by Breuer *et al*[41] in respect of the total and individual bile acid concentrations in the faeces of both control and colon cancer patients. Breuer *et al*[41]

Table 2 *Colon cancer patients (n=10)*

Patient number	*Sex*	*Total bile acids mg/ml*	*DC %*	*C %*	*LC %*	*CDC %*	*UDC %*	*Primary bile acids %*	*Secondary bile acids %*	
14	M	9·64	2	38	3	55	2	93	7	
12	F	50·85	4	38	2	48	8	92	8	
5	F	99·28	8	55	1	37	–	86	14	
18	F	84·02	14	31	3	42	10	78	22	
10	M	46·44	17	38	2	40	3	73	27	median 27%
23	M	71·28	18	38	2	35	7	73	27	
2	M	68·50	21	37	4	36	3	73	27	
16	M	66·79	23	23	7	42	5	66	34	
9	M	8·27	31	32	1	34	2	65	35	
4	M	12·95	37	19	6	37	Trace	56	44	
Mean %		51·80	17·5	34·9	3·1	40·6	4·0	75·5	24·5	
(SD)		(32·30)	(11·2)	(9·8)	(2·0)	(6·6)	(3·4)	(12·0)	(12·0)	

DC – deoxycholic acid; C – cholic acid; LC – lithocholic acid; CDC – chenodeoxycholic acid; UDC – ursodeoxycholic acid.

Table 3 *Control patients (n=8)*

Patient number	*Sex*	*Total bile acids mg/ml*	*DC %*	*C %*	*LC %*	*CDC %*	*UDC %*	*Primary bile acids %*	*Secondary bile acids %*	
33	M	16·28	2	25	4	64	6	90	10	
24	M	60·03	4	49	2	41	4	88	12	
3	M	10·53	4	31	12	49	4	87	13	
31	M	106·45	6	40	2	47	4	87	13	
19	M	10·96	7	34	1	53	5	80	20	
11	M	13·16	16	18	4	58	5	76	24	
32	M	12·05	23	32	3	39	2	71	29	median 27%
29	F	80·77	43	23	3	28	4	50	50	
Mean		38·78	13·1	31·5	3·9	47·4	4·3	78·6	21·4	
(SD)		(38·25)	(14·0)	(9·9)	(3·4)	(11·4)	(1·2)	(13·3)	(13·3)	

DC – deoxycholic acid; C – cholic acid; LC – lithocholic acid; CDC – chenodeoxycholic acid; UDC – ursodeoxycholic acid.

concluded that such results did not refute the hypothesis that bile acids are implicated in the pathogenesis of colorectal cancer but they did not support it. In our view, the wide variations in bile acids traversing the gastrointestinal tract in colon cancer and 'normal' patients, and the lack of significant differences between the distribution of primary and secondary bile acids in these two groups, compliment Breuer *et al*'s[41] findings. We therefore conclude that enterohepatically circulating bile acids are unlikely to play a significant role in the pathogenesis of colon cancer. Given this situation, conditions in which there is a disturbed balance of bile acids distribution in the enterohepatic circulation, such as cholelithiasis or cholecystectomy, are also unlikely to have a significant effect on the incidence of colon cancer *via* that mechanism, and therefore should not predispose to colon cancer.

The inevitable question therefore, is, why have so many authors found a positive association between gall stones and/or cholecystectomy and carcinoma of the large bowel? Careful study of their papers reveals that many of these authors have only found a significant positive association in women, or in patients who subsequently developed carcinomas in the right colon.

The association may result from a simple juxtaposition of two relatively common conditions in affluent communities. Our group, and others, have shown a much higher incidence of gall stones in women than men beyond the age of 50.[24,42] Furthermore, it is becoming apparent that, in these communities, right sided colonic cancers are more common in women, whilst cancers of the left colon and rectum are more common in men.[43] Thus, a random selection of women is more likely to have gallstones than men; similarly a random sample of women is more likely to have right sided colon cancers than men. Because both conditions are relatively common in Western societies, any study which fails to take account of this sex difference will inevitably find an association between cholecystectomy and cancer of the right colon in women.

Unfortunately, the arguments which attempt to link this association causally to enterohepatically circulating bile acids are not supported by consistent findings in analytical studies such as ours, or indeed by consistency in retrospective case control studies, or autopsy studies. Indeed Hill[44] has more recently concluded that bile acids are not initiators of large bowel carcinogenesis but may be involved as promoters of the adenoma carcinoma sequence. If this were the case, then the multiplicity of factors, such as genetic susceptibility, environmental modulation and very long latency, which are known to influence the adenoma carcinoma sequence[45] would explain why there is no consistency in the results of studies which attempt to link bile acids, cholelithiasis or cholecystectomy to the clinical incidence of colorectal cancer in man.

Table 4 *Ratio of primary and secondary bile acids*

Patients	*Primary %*	*Secondary %*
Controls (n=8)	78·6	21·4
Colon cancer (n=10)	75·5	24·5
Gall stones (n=13)	65·5	34·5*

*$p \leq 0.05$ (Wilcoxon test).

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