Retrospective Cohort study

High prevalence of cholestasis, with increased conjugated bile acids in inflammatory bowel diseases patients

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AIM
To investigate the prevalence and causes of cholestasis in patients with inflammatory bowel diseases in the Swiss Inflammatory Bowel Diseases Cohort.

METHODS
A retrospective cohort study was performed of all the patients in the Swiss Inflammatory bowel disease Cohort. Total bile acid was measured for all patients and cholestasis was defined as a concentration > 8 μmol/L. The characteristics of patients with or without cholestasis were compared. Bile acid profiles were then determined for 80 patients with high total bile acid and 80 matched patients with low total bile acid. Bile acid profiles were then compared for smokers vs nonsmokers, ileal vs colonic disease, and inflammatory vs non inflammatory diseases.

RESULTS
Ninety-six patients had more than 8 μmol/L total bile acids.
acid, giving a prevalence of 7.15%. Patients with an obvious cause of cholestasis, such as primary sclerosing cholangitis, were then excluded, leaving 1190 participants with total bile acid < 8 μmol/L and 80 with total bile acid > 8 μmol/L. In multivariate analysis, calcium supplementation was significantly associated with cholestasis (odds ratio, 2.36, 95%CI: 1.00-5.21, P = 0.040) whereas current smoking significantly reduced the risk of cholestasis (odds ratio, 0.42, 95%CI: 0.17-0.91, P = 0.041). Levels of all conjugated bile acids were higher in the cholestasis group than in the control group. When we compared patients with ileal vs colonic disease, the former had higher levels of primary, secondary, and tertiary bile acids whereas patients with colonic disease had higher levels of conjugated bile acids.

**CONCLUSION**

Prevalence of cholestasis is high. Smoking appears to reduce cholestasis. Conjugated bile acids are higher in cholestasis and in colonic disease whereas unconjugated in ileal disease.

**Key words:** Lipid; Liver; Crohn’s disease; Ulcerative colitis; Cholestasis

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Core tip: Inflammatory bowel diseases (IBDs) are often associated with cholestasis. This study shows that the prevalence of cholestasis in IBD patients is high at 7%. Current smoking seems to be a protective factor against cholestasis, maybe reflecting a different gut function than in nonsmokers. Ileal disease was more often associated with elevated non conjugated bile acids levels. Colonic disease was characterized by higher conjugated bile acids levels.

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**INTRODUCTION**

Inflammatory bowel diseases (IBDs) primarily affect the gut, but about 15% of patients also present extra-intestinal manifestations involving the joints, eyes, skin or liver[1]. Flare-ups of the disease are accompanied by an increase in inflammatory markers (such as C-reactive protein) and cholestasis parameters. In some cases, cholestasis may even present as an isolated increase in blood alkaline phosphatase levels found during routine laboratory follow-up. Clinically, cholestasis is characterized by the presence of jaundice, pruritus or signs of malabsorption, whereas biologically, cholestasis is defined as an increased activity of alkaline phosphatase, gamma-glutamyl transpeptidase, conjugated bilirubin or an increased total bile acid level in the blood.

The causes of cholestasis in IBD, either Crohn’s disease (CD) or ulcerative colitis (UC), are multiple. These can range from drug-induced liver toxicity (Azathioprine, nodular regenerative hyperplasia) to specific liver diseases complicating IBD (primary sclerosing cholangitis (PSC), gallstone disease) or para-inflamatory phenomena (TNF-α induced cholestasis)[2].

The proportion of patients with PSC reported in IBD series is about 5% for UC patients and 3.6% for CD patients[3]. The diagnosis of PSC in IBD patients is of the greatest importance because of the high risk of developing colon carcinoma[4] and cholangio-cellular carcinoma. Treatment with ursodesoxycholic acid or 5-ASA have been claimed to decrease the incidence of colon cancer[5].

There are so far no available data on the potential impact of cholestasis on IBD itself. This is an important topic however because cholestasis plays an important role in the malabsorption of liposoluble vitamins, drugs and other liposoluble substances, through the associated decrease in intestinal bile acid concentration and reduced micelle formation. Briefly, after being produced by the liver, primary bile acids – cholic acid and chenodeoxycholate –are glyco- or tauro-conjugated and excreted in the bile into the intestine. At this point, the bile acids are deconjugated by bacteria and transformed into secondary bile acids-deoxycholate and lithocholic acid. Primary and secondary bile acids are then reabsorbed into the entero-hepatic cycle and either glyco- or tauro-conjugated, or transformed into tertiary bile acids-sulfolithocholate and ursodeoxycholate. These pathways could all potentially be altered by IBD. In addition, bile acids are thought to play a role in dysplasia[6], intra-cellular signalling[7] as well as in interactions with the intestinal flora[8].

Since the exact prevalence of cholestasis in IBD patients remains unknown and because cholestasis may have a profound impact on the course of the disease, a fundamental understanding of its aetiologies and clinical relevance are essential.

The primary aim of this study was thus to establish the prevalence of clinical and biological cholestasis in the Swiss IBD Cohort (SIBDC). The secondary aims were to determine the causes of cholestasis and their prevalence as well as to identify bile acid subtypes in confirmed cases of cholestasis.

**MATERIALS AND METHODS**

The SIBDC consists of more than 2800 patients with confirmed IBD, included since 2006 from all over Switzerland. Clinical data on the disease type, endoscopy, evolution, treatments as well as complications are
available as along with serum samples. Data are collected at inclusion and then yearly, during a meeting with the study coordinator. The exclusion criteria of this study were age younger than 16 years and unavailability of serum samples. IBD activity was described using the Crohn’s disease activity index (CDAI) for CD and the Modified Truelove and Witts Index (MTWI) for UC, whereas the site of inflammation was described using the Montreal classification. Cholestasis was defined as a total bile acid (TBA) level higher than 8 μmol/L. In order to measure the prevalence of cholestasis, a TBA assay was performed on all serum specimens using a highly sensitive and specific ELISA test (Labor Eberhard, Dortmund, Germany). Thereafter, a serum bile acid profile was established for all patients with a TBA above 8 μmol/L, and for an equal number of patients adjusted for age and type of disease with a TBA less than 8 μmol/L. These latter patients were randomly selected among those in the SIBDC. This further analysis was performed using ultra-high performance liquid chromatography with tandem mass spectrometry. Serum samples were mixed with a methanolic standard solution. After several centrifugations and dilutions with methanol/water 1:1, the final 20 μL of the supernatant was used for high performance liquid chromatography on a C18 column. The final analyses were performed using a QUATTRO Micro tandem mass spectrometer (Screening-Labor, Hannover, Germany). For a better understanding of the bile acid profiles, Figure 1 shows a diagram that summarizes bile acid metabolism.

**Statistical analysis**

Categorical and continuous variables were described as percentages, means and standard deviations. Groups were compared using Fisher’s exact test for categorical data and the Student t-test for continuous variables. Multivariate analysis was performed using the software R statistics. All variables with \( P < 0.1 \) in univariate analysis were included in multivariate analysis to account for all possible confounders. All significance tests were two-sided, and \( P < 0.05 \) were considered significant. The statistical methods of this study were reviewed by Mr Fournier Nicolas from the SIBDC.

**Ethical considerations**

The study was approved by the ethical review board of the SIBDC and of Geneva University in September 2012. Written, informed consent was obtained from each patient included in the study at inclusion in the SIBDC. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a prior approval by the institution’s human research committee.

**RESULTS**

A total of 1342 patients were included in this study, of which 96 had TBA levels > 8 μmol/L, with a mean level of 21.8 ± 18 μmol/L, and 1246 had TBA levels < 8 μmol/L. The prevalence of cholestasis was thus 7.15%, as defined by elevated bile acids in the SIBDC.

The characteristics of the patients are listed in Table 1. Age, type of disease and activity indexes were similar in the two groups. There were more males in the cholestasis group (63% vs 51%, \( P = 0.027 \)) and nonsmokers (83% vs 72%, \( P = 0.023 \)). Patients in the cholestasis group had lower serum albumin levels (38 vs 39.8 g/L, \( P = 0.016 \)) and higher serum alkaline phosphatase levels (108.1 vs 69.7 U/L, \( P = 10^{-12} \)); a higher proportion received calcium (39% vs 29%, \( P = 0.038 \)), and vitamin E supplements (3% vs 1%, \( P = 0.016 \)), and were treated with tacrolimus (3% vs 1%, \( P = 0.0093 \)) and ursodesoxycholic acid (10% vs 1%, \( P = 2.8 \times 10^{-8} \)). The proportion of patients with PSC was much higher in the cholestasis group (5% vs 1%, \( P = \ldots \))
### Table 1 Clinical characteristics of patients ¹ n (%)  

| Characteristic                  | TBA > 8 (n = 96) | TBA < 8 (n = 1246) | P value |
|--------------------------------|------------------|--------------------|---------|
| **Age (mean ± SD, yr)**        | 44 ± 1.6         | 41.5 ± 0.4        | 0.118   |
| Male                           | 60 (63)          | 639 (51)          | 0.027   |
| Female                         | 35 (36)          | 603 (48)          |         |
| CD                             | 47 (49)          | 716 (57)          | 0.267   |
| UC                             | 45 (47)          | 498 (40)          |         |
| IC                             | 3 (3)            | 26 (2)            |         |
| Disease duration (mean ± SD, yr)| 11 ± 1.2        | 9.8 ± 0.3         | 0.446   |
| CDAI (mean ± SD)               | 92.8 ± 8.7       | 82.1 ± 2.7        | 0.316   |
| MTWI (mean ± SD)               | 3.6 ± 0.6        | 4.1 ± 0.2         | 0.436   |
| Smokers                        | 16 (17)          | 339 (27)          | 0.023   |
| Non smokers                    | 80 (83)          | 903 (72)          |         |
| Extra intestinal manifestations| 21 (22)          | 362 (29)          | 0.133   |
| Arthritis                      | 4 (4)            | 68 (5)            | 0.588   |
| Pyoderma gangrenosum           | 1 (1)            | 12 (1)            | 0.938   |
| Erythema nodosum               | 4 (4)            | 42 (3)            | 0.679   |
| Oral soars                     | 5 (5)            | 70 (6)            | 0.867   |
| Spondylarthritis               | 3 (3)            | 52 (4)            | 0.617   |
| Primary sclerosing cholangitis | 5 (5)            | 10 (1)            | 0.000   |
| Colorectal cancer              | 0 (0)            | 5 (0)             | 0.533   |
| Colonic dysplasia              | 1 (1)            | 5 (0)             | 0.364   |
| Lymphoma                       | 0 (0)            | 0 (0)             | Ns      |
| Osteoporosis                   | 17 (18)          | 184 (15)          | 0.436   |
| Anemia                         | 11 (11)          | 229 (18)          | 0.088   |
| Thrombosis                     | 5 (5)            | 20 (2)            | 0.011   |
| Gallstone                      | 5 (5)            | 33 (3)            | 0.145   |
| Urinary stones                 | 2 (2)            | 38 (3)            | 0.591   |
| Malabsorption                  | 5 (5)            | 38 (3)            | 0.247   |
| **Lab values**                 |                  |                   |         |
| TBA (mean ± SD, μmol/L)        | 21.9 ± 5.5       | 3.1 ± 0.1         | 1.36 × 10⁻⁶ |
| CRP (mean ± SD, mg/L)          | 9.8 ± 1.6        | 11.7 ± 0.7        | 0.460   |
| Ferritine (mean ± SD, μg/L)    | 158.5 ± 39.9     | 239.2 ± 35.9      | 0.516   |
| B12 vitamin (mean ± SD, pmol/L)| 604.1 ± 446.8    | 26.7 ± 21.1       | 0.130   |
| Alkaline phosphatase (mean ± SD, μmol/L) | 108.1 ± 10.8 | 69.7 ± 12 | 10.00 × 10⁻³ |
| Albumin (mean ± SD, g/L)       | 38.0 ± 0.8       | 39.8 ± 0.2        | 0.016   |
| **Medical therapies**          |                  |                   |         |
| Oral 5-ASA                     | 39 (41)          | 415 (33)          | 0.147   |
| Topical 5-ASA                  | 9 (9)            | 114 (9)           | 0.655   |
| Sulfasalazine                  | 0 (0)            | 28 (2)            | Ns      |
| Metronidazole                  | 1 (1)            | 31 (2)            | 0.723   |
| Ciprofloxacin                  | 4 (4)            | 28 (2)            | 0.281   |
| Clarithromycin                 | 0 (0)            | 1 (0)             | Ns      |
| Cyclosporine                   | 0 (0)            | 12 (1)            | Ns      |
| Tacrolimus                     | 3 (3)            | 8 (1)             | 0.009   |
| Azathioprine                   | 38 (40)          | 407 (33)          | 0.177   |
| 6-mercaptopurine               | 5 (5)            | 67 (5)            | Ns      |
| Remicade                       | 9 (9)            | 183 (15)          | 0.174   |
| Humira                         | 1 (1)            | 51 (4)            | 0.172   |
| Cimzia                         | 0 (0)            | 19 (2)            | Ns      |
| Methotrexate                   | 5 (5)            | 79 (6)            | 0.827   |
| Budesonide                     | 9 (9)            | 107 (9)           | 0.708   |
| Systemic prednisone            | 20 (21)          | 170 (14)          | 0.051   |
| Topical prednisone             | 2 (2)            | 28 (2)            | 0.716   |
| Ursodeoxycholic acid           | 10 (10)          | 7 (1)             | 2.84 × 10⁻³ |
| Cholestyramine                 | 2 (2)            | 24 (2)            | 0.708   |
| **Supplements**                |                  |                   |         |
| Iron                           | 12 (13)          | 188 (15)          | 0.492   |
| Vitamin B12                    | 13 (14)          | 191 (15)          | 0.638   |
| Folate                         | 8 (8)            | 125 (10)          | 0.591   |
| Multivitamin                   | 2 (2)            | 76 (6)            | 0.105   |
| Magnesium                      | 6 (6)            | 54 (4)            | 0.381   |
| Calcium                        | 37 (39)          | 356 (29)          | 0.038   |
| Vitamin D                      | 28 (29)          | 270 (22)          | 0.088   |
| Vitamin E                      | 3 (3)            | 9 (1)             | 0.016   |
| Potassium                      | 1 (1)            | 16 (1)            | Ns      |

¹Results expressed in number with percentage in parenthesis otherwise stated. Clinical characteristics of patients with elevated or normal total bile acid level. SD: Standard deviation; CD: Crohn’s disease; UC: Ulcerative colitis; IC: Indeterminate colitis; CDA: Crohn’s disease activity index; MTWI: Modified Truelove and Witts index; TBA: Total bile acid; CRP: C-reactive protein.

0.00007). Multivariate analysis performed at this point showed that PSC (P = 0.012) and tacrolimus treatment (P = 0.017) were the only factors significantly associated with cholestasis.

### Characteristics of patients with the highest TBA concentrations

Sixteen patients had very high TBA levels, between 21 and 483 μmol/L, much higher than those of the other patients. A detailed chart review of these particular patients was performed. The apparent causes for such severe cholestasis were PSC for 2 patients, important clinical and laboratory inflammatory conditions for 2 patients (disease flare with high C reactive protein levels), a complex fistula phenotype for 3 patients, and treatment with azathioprine, methotrexate or tacrolimus for 6 patients. In 25% of these patients the underlying cause of cholestasis remained unexplained after complete investigation.

In order to better define the factors associated with the emergence of cholestasis, we decided to exclude all patients with an obvious cause of cholestasis (PSC, treatment with calcineurin inhibitors, and transplanted patients, who are often treated with these drugs). We therefore removed from the final analysis patients with PSC (n = 5), patients who had undergone organ transplantation (n = 3), patients treated with drugs known to cause cholestasis, namely cyclosporine (n = 12) and tacrolimus (n = 11), and finally patients who had received drugs that can substantially modify the bile acid metabolism (Supplementary Table 1), namely ursodeoxycholic acid (n = 16) and cholestyramine (n = 25) drugs. This yielded a cohort of 1190 patients with a TBA level < 8 μmol/L (93.7%) and 80 patients with a TBA > 8 μmol/L (6.3%). All data considered together, we found that alkaline phosphatase levels, albumin level, supplementation with vitamin D, azathioprine use, current smoking status and previous deep venous thrombosis were significantly associated with cholestasis. There was also a close to significant association with calcium supplementation (P = 0.076). Table 2 shows all the comparison between the groups in univariate analysis.

A multivariate analysis of factors influencing the presence of cholestasis in IBD was then performed (Table 3). We found that supplementation with calcium was significantly associated with cholestasis (OR: 2.36
Table 2  Univariate analysis$^1$ (n (%))

| TBA > 8 | TBA < 8 | $P$ value |
|---------|---------|-----------|
| (n = 80) | (n = 1190) |    |
| TBA (mean ± SD, μmol/L) | 14.66 ± 1.24 | 3.07 ± 0.06 | 6.15 × 10$^{-4}$ |
| Alkaline phosphatase (mean ± SD, U/L) | 81 ± 58.1 | 69.3 ± 1.25 | 0.021 |
| Albumin (mean ± SD, g/L) | 38.0 ± 0.92 | 39.9 ± 0.21 | 0.017 |
| Vitamin D | 25 (31) | 254 (21) | 0.038 |
| Calcium | 30 (38) | 336 (28) | 0.076 |
| Prednisone | 16 (20) | 160 (13) | 0.100 |
| Azathioprine | 38 (46) | 383 (32) | 0.009 |
| Smokers | 12 (15) | 330 (28) | 0.012 |
| Thrombosis | 4 (5) | 17 (1) | 0.015 |

$^1$Results expressed in number with percentage in parenthesis otherwise stated. Univariate analysis of factors differentiating patients with or without cholestasis. TBA: Total bile acid; SD: Standard deviation.

Table 3 Multivariate analysis

| OR  | CI       | $P$ value |
|-----|----------|-----------|
| Gender | 1.48 | 0.83-2.67 | 0.188 |
| Alkaline phosphatase | 1.00 | 0.99-1.01 | 0.282 |
| Albumin | 0.96 | 0.92-1.01 | 0.108 |
| Current smoking status | 0.42 | 0.17-0.91 | 0.041 |
| Azathioprine treatment | 1.57 | 0.87-4.27 | 0.128 |
| Prednisone treatment | 1.15 | 0.53-2.36 | 0.707 |
| Vitamin D supplement | 0.72 | 0.29-1.79 | 0.471 |
| Vitamin E supplement | 0.36 | 0.36-0.34 | 0.110 |
| Calcium supplement | 2.36 | 1.00-5.21 | 0.040 |
| Thrombosis | 1.38 | 0.19-8.59 | 0.701 |

Multivariate analysis of factors associated with a cholestasis comparing 80 patients with cholestasis and 1190 patients without cholestasis. CRP: C-reactive protein; OR: Odd-ratio; CI: Confidence interval.

(1.00-5.21, $P = 0.040$), albeit with a borderline confidence interval, while current smoking status was inversely associated with cholestasis [OR: 0.42 (0.17-0.91), $P = 0.041$].

Bile acids profile

Bile acid subtype profiles were then determined for the group of 80 patients with cholestasis (TBA > 8 μmol/L) and for 80 patients without cholestasis, matched with the former group for age and type of disease. Results of the bile acid profile are fully presented in Table 4. Note that the bile acids are classified as primary, secondary or tertiary bile acids. Table 4 also shows the levels of tauro- and glyco- conjugated bile acids and the primary to secondary and primary to total bile acids ratios, to better characterize the different types present. The levels of 13 of these subtypes were significantly higher in the cholestasis group than in the control group, 3 of which markedly so; specifically, for glycochenodeoxycholate, the levels were 4.72 μmol/L vs 1.42 μmol/L ($P = 1.54 \times 10^{-4}$), for glycodeoxycholate 1.01 μmol/L vs 0.3 μmol/L; $P = 1.39 \times 10^{-5}$, and for tauroursodeoxycholate, 0.2 μmol/L vs 0.05 μmol/L; $P = 8.78 \times 10^{-7}$. Interestingly, these three are respectively primary, secondary and tertiary conjugated bile acids. Overall, we found that the conjugated bile acids were all more elevated in the cholestasis group than in the control group.

Based on these results, we investigated the potential relationship between high TBA levels and three dissimilar factors that can interfere with the metabolism of bile acids, namely the gut microbiota, inflammatory activity, and smoking status. We compared patients with ileal disease to those with colonic disease, patients with high inflammation activity to those without inflammation and active smokers to those who did not smoke. The corresponding univariate analyses are shown in supplementary Table 2.

Patients with ileal disease were more likely to receive vitamin D and B12 supplementation than those with colonic disease were (35% vs 20%, $P = 0.025$ and 26% vs 5%, $P = 0.0001$, respectively). They were also more likely to be treated with certolizumab pegol and budesonide (3% vs 0%, $P = 0.041$, and 18% vs 7%, $P = 0.027$, respectively), and less likely to be treated with oral or topical SASA (53% vs 15%, $P = 5.8 \times 10^{-7}$, and 11% vs 0%, $P = 0.004$, respectively). There was a marked difference in the proportion of smokers in the two groups, with, 90% of patients with colonic disease being nonsmokers vs just 66% in the ileal disease group ($P = 0.0001$).

Patients with active IBD had a higher UC activity index (8.3 ± 1.2 vs 3.3 ± 0.5, $P = 0.0001$) while the CD activity index was not different between the two groups. Patients with active IBD were more likely to have anemia (34% vs 11%, $P = 0.0007$), to have lower albumin levels (35.6 ± 1.3 vs 40.2 ± 0.6, $P = 0.001$) and were more often treated with metronidazole (5% vs 0%, $P = 0.014$).

Patients who were active smokers were more likely to suffer from CD (81% vs 48%, $P = 0.003$), to have more frequent pulmonary embolism (6% vs 0%, $P = 0.038$) but were less likely to receive systemic steroids (9% vs 27%, $P = 0.036$).

The differences in bile acid subtypes are illustrated in Figure 2. Interestingly, there are several significant differences in terms of the site of bowel inflammation (ileal vs colonic). Patients in the ileal disease group were indeed found to have more primary, secondary and tertiary bile acids, specifically higher chenodeoxycholate levels (2.41 μmol/L vs 0.59 μmol/L, $P = 3.3 \times 10^{-5}$), primary to TBA ratios (0.43 vs 0.15, $P = 7.8 \times 10^{-5}$), deoxycholate levels (1.34 μmol/L vs 0.41 μmol/L, $P = 0.001$) and ursodeoxycholate levels (0.37 μmol/L vs 0.13 μmol/L, $P = 1.3 \times 10^{-5}$). On the contrary, patients in the colonic disease group had higher concentration of conjugated bile acids namely glycodeoxycholate (1.42 μmol/L vs 0.6 μmol/L, $P = 0.006$), taurocholate (0.3 μmol/L vs 0.07 μmol/L, $P = 0.008$), taurochenodeoxycholate (0.62 μmol/L vs 0.24 μmol/L, $P = 0.001$) and taurodeoxycholate (0.17 μmol/L vs 0.06 μmol/L, $P = 0.0008$), all of which are either primary or secondary...
Table 4  Bile acids subtypes

| TBA > 8 (80) | TBA < 8 (80) | P value |
|-------------|-------------|---------|
| Primary | 14.64 (1.24) | 3.31 (0.22) | 1.07 × 10^-10 |
| CA | 1.90 (0.92) | 2.09 (0.96) | 0.008 |
| Cholic acid | 3.78 (1.76) | 2.96 (1.37) | 0.009 |
| CDC chenodeoxycholate | 7.00 (1.37) | 2.96 (1.37) | 0.002 |
| Secondary | 2.06 (0.41) | 0.68 (0.13) | 0.002 |
| DC deoxycholate | 1.35 (0.27) | 0.28 (0.04) | 0.000 |
| LCA lithocholic acid | 0.07 (0.01) | 0.07 (0.01) | 0.9 |
| Tertiary | 0.34 (0.05) | 0.14 (0.03) | 0.000 |
| UDC ursocholate | 0.37 (0.08) | 0.04 (0.01) | 0.000 |
| Primary conjugated | 1.78 (0.28) | 0.38 (0.05) | 1.83 × 10^-7 |
| GCA glycocholate acid | 0.74 (0.11) | 0.18 (0.02) | 1.58 × 10^-6 |
| TCDC taurochenodeoxycholate | 4.72 (0.33) | 1.42 (0.12) | 1.54 × 10^-10 |
| GDC glycodeoxycholate | 1.01 (0.10) | 0.30 (0.04) | 1.38 × 10^-8 |
| Secondary conjugated | 0.20 (0.05) | 0.05 (0.01) | 8.78 × 10^-10 |
| GDCD taurochenodeoxycholate | 0.04 (0.01) | 0.01 (0.00) | 8.46 × 10^-7 |
| GLC glycochenodeoxycholate | 0.09 (0.01) | 0.09 (0.01) | 0.646 |
| Tertiary conjugated | 0.06 (0.01) | 0.00 (0.00) | 1.69 × 10^-8 |
| UDCD tauroursodeoxycholate | 0.84 (0.12) | 0.19 (0.03) | 4.58 × 10^-10 |
| Hydroxides | 0.94 (0.02) | 0.01 (0.00) | 0.291 |
| DHA dihydroxycholesenteric acid | 0.001 (0.001) | 0.000 (0.000) | 0.275 |
| THCA trihydroxycholesenteric acid | 3.48 (0.92) | 4.42 (1.34) | 0.566 |
| Primary/secondary | 0.23 (0.05) | 0.31 (0.04) | 0.211 |
| Conjugated glycine/taurine | 11.64 (1.18) | 10.70 (0.89) | 0.537 |
| Primary | 6.40 (0.61) | 5.15 (0.66) | 0.209 |

Bile acids subtypes expressed in μmol/L with standard deviation under bracket, in 80 patients with a total bile acids level of more than 8 μmol/L, compared with 80 patients with a total bile acids level of less than 8 μmol/L.

DISCUSSION

In this study, cholestasis was observed in 7.15% of patients in the SIBDC. As expected, the patients with the most severe cholestasis were those with PSC and those treated with a drug known to induce cholestasis.

We were surprised to find a low prevalence of PSC in our cohort (1.1%) compared with the values previously reported in the literature that range from 3.6% for CD to 5% for UC[13]. This might influence the prevalence of cholestasis in our cohort as PSC is one of the most important causes of cholestasis in IBD. One explanation could be that there are more patients with CD than with UC in our SIBDC cohort. We assumed that each case of PSC included in the central registry had been fully verified; however, we cannot exclude the possibility that some cases of PSC may have been missed.

To highlight factors more rarely associated with cholestasis, we chose to compare patients with and without cholestasis after having excluded those with the most common causes. Patients with the highest TBA levels (for example those with PSC or treatment with tacrolimus) were thereby removed from the analysis, making it less powerful. This decision was made to allow a more detailed analysis of the causes of moderate cholestasis, which would otherwise have remained unexplained.

Although some of the causes of cholestasis were expected in certain patients, notably those with higher alkaline phosphatase activity, lower albumin level and increased vitamin D supplementation because of disturbed lipid absorption, some of the other causes identified were more surprising. In particular, we found that patients with cholestasis were more likely to have been exposed to thiopurines than those in the control group. This class of drug frequently causes liver adverse events, including transient elevation of transaminases, or more rarely, nodular regenerative hyperplasia changes[13]. Thiopurine-induced cholestasis has seldom been described[14].

Another interesting point was that active smoking was protective against the development of cholestasis. Because smoking is a well-known risk factor for disease activity in CD, we would anticipate on the contrary that any increased inflammatory activity would lead to more pronounced cholestasis in smokers.

We found that nonsmokers had different bile acid patterns from smokers, with higher taurocholate levels, a secondary conjugated bile acid, indicating that this bile acid is better reabsorbed and then conjugated by the liver and suggesting that nonsmokers’ intestines are probably more functional. Since stopping smoking has been shown to have an impact on gut microbiota[15], we speculate that smoking may affect the way in which the microbiota transform bile acids in the intestine.

Calcium supplementation was associated in multivariate analysis with the presence of cholestasis, although this association was only just statistically significant. It is true that calcium supplementation may...
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Figure 2 Representation of bile acid subtypes in 3 different situations: ileal vs colonic disease; inflammatory vs non-inflammatory disease and smokers and non-smokers. TBA: Total bile acids; CA: Cholic acid; CDC: Chenodeoxycholate; DC: Deoxycholate; LCA: Lithocholic acid; UDC: Ursodeoxycholate; TC: Taurocholate; GCA: Glycocholate acid; TDC: Taurochenodeoxycholate; GDC: Glycochenodeoxycholate; TDC: Taurodeoxycholate; TLC: Taurolithocholate; GLC: Glycolithocholate; TUDC: Tauroursodeoxycholate; GUDC: Glycoursodeoxycholate; DHCA: Dihydroxycholestanic acid; THCA: Trihydroxycholestanic acid; CA + CDC/DC + LCA: Primary bile acids on secondary bile acids ratio; CA + CDC/TBA: Primary bile acids on TBA ratio; GCA + GCDC/TC + TCDC: Conjugated primary bile acids with glycine on taurine ratio; GDC + GLC/TDC + TLC: Conjugated secondary bile acids with glycine on taurine ratio. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
be a surrogate marker for inflammatory flares of the disease because it is often prescribed in conjunction with prednisone. It is noteworthy that calcium supplementation has also recently been shown to modify the composition of stool and the content of bile acids.

Looking at the bile acid subtypes that characterized cholestasis in these patients, we found that patients with cholestasis had higher levels of all the conjugated bile acids. Looking in detail at each subtype, we found that patients with and without cholestasis differed mainly in terms of their levels of glycochenodeoxycholate, glycodeoxycholate and tauroursodeoxycholate, which are conjugated bile acids, respectively primary, secondary and tertiary. This suggests that the liver plays a role in pathogenesis. Similarly, Gnewuch et al. showed that UC patients with hepatobiliary manifestations had increased primary and secondary conjugated bile acid levels. Denk et al. showed in a rat model of cholestasis that the best anti-cholestatic effect was obtained with conjugated ursodeoxycholate, making hepatic conjugation an essential pathway against cholestasis.

The bile acid subtype profiles of patients with ileal disease were very different from those of patients with colonic disease, with all the primary, secondary and tertiary bile acids being found in higher concentrations in the former. This data strongly supports the role of an altered microbiota and absorption in ileal disease. Bile acids are deconjugated and transformed into secondary bile acids, but are less well absorbed in this form. Conversely, we found that primary and secondary conjugated bile acids were significantly reduced in patients with ileal involvement, most probably because of gut malabsorption, a condition already reported in patients with ileal resection by Gnewuch et al. Similarly, we identified an increased glycine to taurine conjugation ratio in patients with ileal involvement, which has also been reported previously in patients with ileal diseases.

Patients with high inflammation activity had lower levels of glycochenodeoxycholate, a primary conjugated bile acid, than those with a low or no inflammation activity did, most probably because of disturbed intestinal absorption due to inflammation. Both primary and secondary bile acids have to be reabsorbed in order to be conjugated once more in the liver. This data is consistent with previous findings that demonstrated the presence of bile acid dysmetabolism in the stools of IBD patients and lower levels of secondary bile acids in their sera.

The main strengths of our study are the number of patients included and the richness of the data in the SIBDC, which is the largest IBD cohort in Switzerland. The main weakness of our findings is the absence of any correlation with gut microbiota, which seems to play a significant role in bile acid metabolism in these patients.

In conclusion, our study reports the prevalence of cholestasis in IBD patients for the first time, with 7% of the cohort being affected. Current smoking activity seems to be a protective factor against cholestasis. However, this may only reflect the effect of smoking on the intestinal inflammatory disease. Regarding bile acid subtype patterns, conjugated bile acids were more elevated in patients with cholestasis, suggesting an intrahepatic process. Interestingly, ileal disease was more often associated with elevated primary, secondary and tertiary bile acids, suggesting bacterial involvement, whereas a colonic disease was characterized by higher levels of conjugated bile acids.

**ARTICLE HIGHLIGHTS**

**Research background**

Inflammatory bowel diseases (IBDs) are often associated with cholestasis, which can be caused by inflammation, drugs, or extra-intestinal manifestations. There is a lack of data in the literature on the prevalence and characteristics of cholestasis.

**Research motivation**

Measuring cholestasis and its prevalence and describing its characteristics and the main associated factors should improve our understanding of its causes and help to prevent or avoid the development of cholestasis in IBD.

**Research objectives**

The main objective of this study was to establish the prevalence of clinical and biological cholestasis. The secondary objective was then to clarify the causes and characteristics of cholestasis by studying the corresponding bile acid subtypes.

**Research methods**

A total of 1342 patients from the Swiss Inflammatory Bowel Disease Cohort were included and cholestasis was measured via their total bile acid levels. Patients with the main well-known causes of cholestasis (such as primary sclerosing cholangitis for example) were then excluded to study less obvious causes. The concentrations of different bile acid subtypes were measured in two groups of patients, either with or without cholestasis. The bile acid profiles of patients in the two groups were compared for different clinical situations, notably ileal vs colonic disease and for smokers vs nonsmokers.

**Research results**

The prevalence of cholestasis was found to be high, at 7.15%. Multivariate analysis showed that calcium supplementation was significantly associated with cholestasis whereas current smoking significantly reduced the risk of cholestasis. Regarding bile acid subtypes, patients in the cholestasis group had higher levels of all conjugated bile acids than those in the control group did. Comparing patients with ileal disease with those with colonic disease, the former had higher levels of unconjugated bile acids while the latter had higher levels of conjugated bile acids. These results highlight the probable role played by gut bacteria and the liver in the different types of cholestasis. What exactly these roles are should now be studied in more detail.

**Research conclusions**

This study found that the prevalence of cholestasis is high in patients with IBD. Different subtypes of bile acid were associated with cholestasis, colonic disease, and ileal disease, indicating probable interactions between the gut microbiota, the liver and the different types of IBD. As expected, ileal or colonic involvement, smoking status and inflammation activity all modified the type of cholestasis presented by patients. A prospective analysis of cholestasis and its characteristics should be performed to improve our understanding of this condition and develop prevention strategies.

**Research perspectives**

Cholestasis is frequent in IBD. The characteristics of cholestasis vary with the type of IBD and inflammatory activity. Cholestasis should now be studied prospectively to better understand how the gut microbiota and the liver are involved.
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