Roles of Sphincter of Oddi Laxity in Bile Duct Microenvironment in Patients with Cholangiolithiasis: From the Perspective of the Microbiome and Metabolome

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BACKGROUND: Bile duct microenvironment plays several key roles in cholangiolithiasis occurrence. Sphincter of Oddi laxity (SOL) is associated with cholangiolithiasis, probably due to enhanced reflux of intestinal contents that changes the microenvironment. However, the microenvironment has not been investigated comprehensively.

STUDY DESIGN: Patients with cholangiolithiasis were consecutively recruited and their bile was collected intraoperatively for high-throughput experiments. Pyrosequencing of 16S ribosomal RNA gene was performed to characterize the microbiota in the bile. A liquid chromatography mass spectrometry-based method was used to profile bile composition. Clinical manifestation, microbiome, and bile composition were compared between patients with and without SOL.

RESULTS: Eighteen patients with SOL and 27 patients without SOL were finally included. Patients with SOL showed more severe inflammation. Bacteria in the bile duct were overwhelmingly aerobes and facultative anaerobes. *Proteobacteria* and *Firmicutes* were the most widespread phylotypes, especially *Enterobacteriaceae*. Compared with those without SOL, patients with SOL possessed more varied microbiota. In the SOL group, pathobionts, such as *Bilophila* and *Shewanella algae* had richer communities, and harmless bacteria were reduced. Metabolomics analysis showed the differences in bile composition between groups were mainly distributed in lipids and bile acids. Particularly, the increased abundance of *Bilophila* involved in taurine metabolism was associated with reduced contents of taurine derivatives in the bile of patients with SOL.

CONCLUSIONS: A bile duct microenvironment with more severe bacterial infection and stronger lithogenicity was found in patients with SOL. The findings suggest a possible mechanism of cholangiolithiasis and provide the basis for future strategies for prevention of cholangiolithiasis recurrence. (J Am Coll Surg 2016;222:269−280. © 2016 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

Cholangiolithiasis is an endemic disease highly prevalent in the Far East, including China, South Korea, Japan, and Southeast Asia.[1] It has been reported that more than one-third of the population in these regions suffer from this disease, and <5% in the West are affected. However, Western countries also face increasing cases of this condition due to immigration from the Far East.[2] Cholangiolithiasis can cause bile duct stricture and infection and result in liver abscess, hepatatrophia, liver cirrhosis, and even cholangiocarcinoma.[3] The pathophysiology of cholangiolithiasis is not well understood due to the complicated factors involved. Epidemiologically, race, diet, and sanitary conditions are associated with incidence of cholangiolithiasis, and pathophysiologically, changes in

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Abbreviations and Acronyms

LC-MS = liquid chromatography mass spectrometry
OTU = operational taxonomic unit
rRNA = ribosomal RNA
SOL = sphincter of Oddi laxity
TIC = total ion chromatogram
VIP = variable importance in projection

microenvironment, such as bacterial infection, cholestasis, and metabolic disorders, are believed to contribute to calculi formation. Although many hypotheses about formation of calcium bilirubinate, the predominant component of the bile duct stone rather than gallbladder stone, have been proposed, the exact pathogenesis remains unclear.

Changes in the local microenvironment are the key steps of calculi formation. Cholangiolithiasis tends to recur probably because current treatments focus attention on removing the calculi but ignore the need to correct the microenvironment. Sphincter of Oddi, which controls the unidirectional outflow of bile and separates the bile duct from the bacteria-filled intestinal tract, is thought to be a gatekeeper of the almost-sterile biliary tract. Dysfunction of the sphincter of Oddi can lead to dramatic variation of the bile duct microenvironment. In our previous study, we found that roughly one-half of patients with cholangiolithiasis had sphincter of Oddi laxity (SOL), and these patients exhibited higher risks of recurrence and reoperation,[4] suggesting an association between cholangiolithiasis and SOL. Intriguingly, SOL appears to be much more prevalent in Asia, as very few reports are available from Western countries. Regurgitation of the duodenal contents under the condition of SOL is evident and can result in a contaminated and inflammatory environment that facilitates lithogenesis. However, details such as changes of microbiota and bile composition in the bile duct have not been investigated.

As critical components of the bile duct microenvironment, the bile duct microbiota and bile compositions play key roles in cholangiolithiasis. Previous studies using culture-dependent methods have identified many potential pathogens associated with biliary infection, and the changes in microenvironments of patients with SOL are poorly understood. In addition, most bacteria are not clinically culturable, and only a few bile compositions have been analyzed to date. Currently, novel techniques with high throughput and high resolution provide a more comprehensive and in-depth understanding of microbial community and compositions in various types of human samples, including feces, serum, urine, and tissue.[5,6]

In the current study, we performed high-throughput MiSeq pyrosequencing targeting the 16S ribosomal RNA (rRNA) V3 and V4 regions and reverse-phase liquid chromatography with quadruple time-of-flight mass spectrometry to characterize the bile duct microenvironment in patients with cholangiolithiasis and compare the differences between patients with and without SOL. This is the first study on bile duct microenvironment using a method integrating microbiome and metabolome. Our findings depicted a novel picture of the bile duct microenvironment in cholangiolithiasis with respect to sphincter of Oddi laxity, aiming to deepen our understanding of bile duct calculi formation.

METHODS

Patients and samples

This study was appropriately approved by the ethics committee of our hospital. Patients with cholangiolithiasis were consecutively included from February 2014 to January 2015. Cholangiolithiasis was diagnosed preoperatively by imaging evidences, and confirmed intraoperatively by surgical findings. Patients with hepatobiliary tumors, congenital biliary malformations, biliary tract reconstruction, hepatic cirrhosis, and pancreatitis were excluded. SOL was intraoperatively diagnosed if a choledochoscope (Olympus CHF P20, external diameter = 4.9 mm) could smoothly reach the duodenum via the sphincter of Oddi without any dilation procedures. Demographic and clinical data were collected prospectively. Informed written consents were obtained from all patients. Bile samples were extracted from the supraduodenal segment of the common bile duct with a 5-mL germ-free injector before any invasive manipulation on the bile duct occurred, and stored immediately in liquid nitrogen. The samples were then kept at −80°C until processed.

DNA extraction and MiSeq pyrosequencing of 16S ribosomal RNA gene amplicons

DNA was purified and stored by standard methods.[7] Universal primers (forward: 5′-GTACTCCTACGGGAGG CAGCA-3′, reverse: 5′-GTGGACTACHVGGGTWTC TAAT-3′) with 8-nt barcodes were used to amplify the V3 and V4 hypervariable regions of 16S rRNA genes for sequencing using a MiSeq sequencer.[8,9] Polymerase chain reaction was performed regularly in triplicate for each sample. The products of the same sample were combined together and subjected to electrophoresis. The correct-size DNA was purified using Gel Extraction Kit (Omega Bio-tek) and quantified with Qubit (Life Technologies). The sequencing library was prepared using