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Comparison between the Sinus and Gut Microbiome in Patients with Chronic Sinus Disease

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Abstract

Objectives: Associations between the gut microbiome and various non-GI related diseases have been detailed in recent studies. This investigation aims to directly compare the gut and sinus microbes in patients with chronic sinus disease and in control patients to determine if any link exists between the sinus and gut microbiota.

Methods: This was a prospective study conducted from February 2016 to August 2017. It was conducted at a tertiary care academic rhinology practice on 16 patients undergoing rhinologic surgery. The primary outcome measure was to determine if any overlap exists between the gut and sinus microbiome in a given subject. A secondary outcome was to assess the effect of prior antibiotic therapy on the diversity of the gut microbiome.

Results: There were 7 patients with chronic rhinosinusitis (CRS) with polyps, 6 patients with CRS without polyps and 3 control patients. Only one patient demonstrated an overlap of sinus and gut microbiotia. In patients receiving a reduced number of antibiotic courses over the past 24 months (0 or 1 course), there was a mean of 7.7 (SD 2.2) gut bacteria isolated from stool samples. In patients receiving more antibiotic courses (2+ courses), there was a mean of 5.1 (SD 2.3) gut bacteria isolated. This difference reached statistical significance (p=0.043).

Conclusion: Minimal overlap between the sinus and gut microbiome was demonstrated, but further studies are needed to elucidate this potential association. This study supports the theory that antibiotics tend to reduce microbial diversity in the gastrointestinal tract.

Keywords: Anti-bacterial agents; Biodiversity; Sinusitis/microbiology; Gastrointestinal tract/microbiology; Bacteria/classification

Introduction

Chronic rhinosinusitis (CRS) with or without nasal polyposis is a prevalent disease that affects approximately 16% of the US population. It is a condition that has a significant negative impact on quality of life and leads to massive health care expenditures [1]. Until recently, the microbiota underlying the disease has been poorly elucidated. Advances that have been made are based on a shift in the identification process away from simple culture, which failed to recognize the majority of species present. Contemporary techniques include 16S rRNA gene sequencing, FISH and quantitative PCR, which are superior in the detection of the sparser bacterial taxa [2-4]. Our understanding of the organismal diversity comprising the sinus microbiome vastly increased with such techniques.

Likewise, knowledge regarding the microbiome of the digestive tract has been rapidly increasing in recent years. Variations in the gut microbiome have been linked to diseases including renal failure [5], type 2 diabetes [6] and obesity [7,8]. As knowledge of the profound influence of the gut microbiota continues to expand, researchers are also seeking to find associations between the gut microbiome and diseases that have not been thought to be nutritional-related, such as autism spectrum disorder [9].

Associations between gastrointestinal and rhinologic conditions have been highlighted by various studies including those that demonstrate a higher incidence of CRS in patients with inflammatory bowel disease [10]. Yang et al. [11] demonstrated high levels of staphylococcus enterotoxin B in both the sinus and gut mucosa in patients with ulcerative colitis. They proposed that patients with CRS swallow *staphylococcus* enterotoxin B originating from sinusitis and these pathogens initiate an inflammatory cascade in the gut that progresses to ulcerative colitis. Additionally, a link between gastroesophageal reflux disease and rhinosinusitis has been hypothesized based on studies showing a presence of *H. pylori* in sinus cultures of patients with CRS [12,13].

To date, no studies have been performed that directly compare the sinus and digestive tract microbiomes. Recent discussion has also been devoted to the effects of systemic antibiotics on the gut microbiome. Initial studies have shown that antibiotic courses can alter the composition of the gut microbiome even years following treatment. However, individualized responses can be highly varied [14,15]. A second goal of this study is to analyze the effect of antibiotics administered for CRS on the composition of the gut microbiome.

Materials and Methods

The University of Florida Institutional Review Board granted research ethics approval. This study was conducted at a tertiary care academic referral center.

Study design and setting

The design is a prospective study with data collected from February 1, 2016 to November 1, 2017. Patients were registered for the study at a tertiary care rhinology practice at the University of Florida.

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Study groups and patient selection

There were 3 subsets of patients. The first subset consisted of patients with chronic rhinosinusitis with nasal polyps. The second subset consisted of patients with chronic rhinosinusitis without nasal polyps. The third subset served as the control group and consisted of patients who present with rhinologic problems that are not related to chronic rhinosinusitis.

All patients >18 years old who underwent rhinologic surgery were considered for the study. Patients with CRS defined by symptom, endoscopic and radiographic criteria [16] were identified by the investigators. This was defined as purulence in the middle meatus, presence of nasal polyps or radiographic evidence of paranasal sinus inflammation as well as 12 or more weeks of two of the following four symptoms: mucopurulent drainage, nasal obstruction, facial pain/pressure and decreased sense of smell. The presence or lack of nasal polyposis was determined by endoscopic evaluation. Control patients consisted of patients who presented to the practice with pathology such as septal deviation, cerebrospinal fluid leak, turbinate hypertrophy, concha bullosa or unilateral masses that did not have radiologic, endoscopic or clinical evidence of CRS.

Data collection

Patient interviews were conducted to obtain data such as demographic factors, previous surgeries, previous antibiotic treatment, history of GERD and history of GI pathology. Gender was self-reported. Stool samples were swabbed by the patient within 24 h of surgery and brought to the investigators on their operative dates. Patients were instructed to refrigerate the stool samples after collection. During operations, sinus samples were swabbed from the nasal floor, middle meatus and sphenoethmoidal recess. Both stool and sinus samples were subjected to DNA extraction and quantitative PCR with DNA sequencing (MicroGen DX Laboratory, Lubbock, Texas).

DNA sequencing

Per MicroGen DX protocol, the Ion Torrent PGM sequencer was used to determine which microbes were present. Forward and reverse primers were utilized to detect the target sequence in each sample and amplify it. The samples were differentiated from one another when run on the Ion Torrent sequencer by a unique identifying sequence ("tag") attached to the forward primer. The tag was implemented when the targeted sequence was amplified using PCR. The PCR comprised a 5 min denaturation step at 95°C, 35 cycles of 94°C for 30 s 52°C for 40 s and 72°C for 60 s. Next, there was a final extension step of 72°C for 10 min. After amplification, the sample DNA was grouped together based on amplification strength for downstream applications. Purification of DNA was then performed by removing small fragments using the Qiagen Minelute kit and Agencourt Ampure beads. The DNA was then introduced to the emulsion PCR. This attached one DNA strand to one Ion Sphere Particle. Amplification of the DNA then occurred and the one Ion Sphere Particle held several copies of the DNA strand. The DNA Ion Sphere Particles were then retrieved, enriched and prepared for sequencing. Next, the beads were run on the Ion Torrent sequencer. This sequencer uses DNA polymerase to integrate phosphorylated dNTPs into the DNA strand. When each dNTP is incorporated, a hydrogen ion is released. The ion triggers a change in pH proportional to the number of nucleotides incorporated in the DNA strand. This is captured by the sequencer's sensing layer inside the chip. The sequencing reads are then analyzed and any of them which fall below the baseline quality score or appropriate length are discarded. The high-quality reads are then reported to the investigators by Microgen DX.

Statistical analysis

Student's t-test was used to compare the numbers of gut bacteria/fungi between patients. A level of statistical significance was set as $p<0.05$.

Results

There were 16 patients who met the study criteria and agreed to participate. Seven patients had CRS with polyps (CRScP), 6 had CRS without polyps (CRSsP) and 3 were control patients. One control patient underwent surgery for inferior turbinate reduction and the other two for unilateral sinus masses. None of the three had endoscopic or radiologic evidence of chronic rhinosinusitis. Table 1 details the demographics of the three groups as well as other related medical conditions i.e. allergic rhinitis, gastroesophageal reflux disease and other gastrointenstinal related diagnoses such as inflammatory bowel disease. None of the patients in the study carried a GI-related diagnosis other than GERD. The presence of GERD was determined based on prior diagnosis by other physicians. The presence of allergic rhinitis was based on positive allergy testing.

Regarding the number of antibiotic courses the patients received, they were divided into two groups. The first group received 0 or 1 antibiotic course in the past year, defined as an oral or IV antibiotic spanning five or more days. The second group received 2 or more antibiotic courses in the past year. Table 2 details these groups. The first group received an average of 0.44 antibiotic courses in the prior year and the second group received an average of 3.7 antibiotic courses in the prior year.

	CRS without polyps	CRS with polyps	Control
Number of patients	6	7	3
Mean age (years)	53.0	51.7	65.0
Males	0	7	2
Females	6	0	1
Allergic rhinitis	6 (100%)	6 (85.7%)	1 (33.3%)
GERD	3 (50%)	2 (28.6%)	0 (0%)
Previous sinus surgeries (mean)	1.2	1.7	0
Previous GI surgeries	0	0	0
Any other GI Dx (IBD, etc.)	0	0	0
ABX courses last 12 months (mean)	2.7	2.0	0

Abbreviations: CRS: Chronic Rhinosinusitis; GERD: Gastroesophageal Reflux Disease; DX: Diagnosis; IBD: Inflammatory Bowel Disease; ABX: Antibiotic

Table 1: Demographics and medical history, broken down by disease status.

	0-1 ABX	2+ ABX
Number of patients	9	7
Mean age (years)	56.3	52.6
Males	5	4
Females	4	3
Allergic rhinitis	5 (55.6%)	7 (100%)
GERD	2 (33.3%)	4 (57.1%)
Previous sinus surgeries (mean)	0.33	2.3
Previous GI surgeries	0	0
Any GI Dx (IBD, etc.)	0	0
ABX courses last 12 months (mean)	0.44	3.7

Abbreviations: ABX: Antibiotic; GERD: Gastroesophageal Reflux Disease; DX: Diagnosis; IBD: Inflammatory Bowel Disease

Table 2: Demographics and medical history, broken down by antibiotics received.

Tables 3-5 reveal the number of different species of bacteria found in the sinus and gut for each patient, as well as the predominant organisms isolated. There was only one patient who demonstrated any overlap between the sinus and gut bacteria. One patient in the CRScP group shows a predominance of *Pseudomonas aeruginosa* in the sinus as well as in the stool.

Table 6 compares the number of gut bacteria and fungi found in the stool based on antibiotics received. Overall, the group receiving fewer antibiotic courses had significantly more stool bacteria isolated (7.6 [SD 2.2] versus 5.1 [SD 2.3], p=0.043). There was no significant difference between the two groups in the number of gut fungi isolated (p=0.88).

Discussion

Though links between the sinus and gut microbiome have been proposed in the literature, our patient set did not demonstrate a notable link. Only one of the patients in our study exhibited an overlap between a microbe in the sinus and gut. *Pseudomonas aeruginosa* was the predominant organism isolated in both the sinus and gut of this patient with CRS. In no other patients in this study was *Pseudomonas* evident in the stool. Ohara et al. [17] analyzed associations with *Pseudomonas* in the GI tract and found that prior antibiotic use demonstrated the strongest association. While the above patient's use of antibiotics may have contributed to *Pseudomonas* colonization in the gut, another postulation is that sinusitis-mediated *Pseduomonas* was swallowed by the patient which traveled to the GI tract and colonized. One study indeed has shown that ingested *Pseudomonas aeruginosa* results in isolation of the bacteria in the stool [18].

Our analysis of the relation between antibiotics and stool microbes finds that the number of bacterial species isolated from the stool increases with less exposure to antibiotics. Dethlefsen et al. [15] studied the effects of ciprofloxacin on the GI microbiome of three individuals. They also found a reduction in microbial diversity after exposure to antibiotics. While their findings showed a recovery in diversity by 4 weeks, some bacterial taxa remained affected for 6 months. A similar study regarding a 7 day course of clindamycin demonstrated an impact on GI microbial diversity up to 2 years following administration of the antibiotic [19]. Our study supports the theory that systemic antibiotics lead to a decrease in diversity in the gut microbiome.

The effects of a loss in diversity have been shown in recent studies utilizing animal models. One mouse model demonstrated a loss in GI bacterial diversity increased susceptibility to *Clostridium dificile* infections [20]. An additional mouse model exhibited partial ablation of gut microbiota increased the rate of development of diabetes mellitus [21]. Also proposed are links between a less diverse GI microbiome and dermatologic conditions, atopic diseases, immune conditions and variations in the metabolism of drugs [22-24]. It has also been

	Sinus bacterial species (predominant organism)	Stool bacterial species (predominant organism)	Overlap
CRSsP1 (pan)	1 (*Pseudomonas aeruginosa*)	6 (*Bacteroides fragilis*)	None
CRSsP2 (pan)	1 (*Staphylococcus schleferi*)	5 (*Escherichia coli*)	None
CRSsP3 (pan)	1 (*Staphylococcus epidermidis*)	5 (*Escherichia coli*)	None
CRSsP4 (eth, sph)	0	11 (*Streptococcus parasanuinis*)	None
CRSsP5 (max, sph)	10 (*Corynebacterium tuberculostearicum*)	6 (*Escherichia coli*)	None
CRSsP6 (max, eth)	1 (*Staphylococcus epidermidis*)	5 (*Escherichia coli*)	None

Abbreviations: CRSsP: Chronic Rhinosinusitis without Polyps; pan: Pansinusitis; eth: Ethmoidal Sinusitis; sph: Sphenoidal Sinusitis; max: Maxillary Sinusitis

Table 3: Number of bacterial species isolated in each CRSsP patient.

	Sinus bacterial species (predominant organism)	Stool bacterial species (predominant organism)	Overlap
CRScP1 (pan)	4 (*Propionibacterium acnes*)	4 (*Escherichia coli*)	None
CRScP2 (pan)	1 (*Pseudomonas aeruginosa*)	8 (*Enterobacter hormaechei*)	None
CRScP3 (pan)	4 (*Propionibacterium acnes*)	1 (*Escherichia coli*)	None
CRScP4 (pan)	2 (*Propionibacterium acnes*)	7 (*Provotella copri*)	None
CRScP5 (pan)	1 (*Klebsiella pneumoniae*)	5 (*Bacteroides fragilis*)	None
CRScP6 (pan)	1 (*Pseudomonas aeruginosa*)	8 (*Pseudomonas aeruginosa*)	1
CRScP7 (pan)	2 (*Staphylococcus aureus*)	7 (*Escherichia coli*)	None

Abbreviations: CRScP: Chronic Rhinosinusitis with Polyps; pan: Pansinusitis

Table 4: Number of bacterial species isolated in each CRScP patient.

	Sinus bacterial species (predominant organism)	Stool bacterial species (predominant organism)	Overlap
Control 1 (mass)	4 (*Staphylococcus epidermidis*)	10 (*Bacteroides stercoris*)	None
Control 2 (mass)	1 (*Enterobacter aerogenes*)	10 (*Escherichia coli*)	None
Control 3 (ITR)	4 (*Haemophilis influenzae*)	7 (*Enterobacter hormaechei*)	None

Abbreviations: ITR: Inferior Turbinate Reduction

Table 5: Number of bacterial species isolated in each control patient.

	0-1 ABX (mean 0.44 courses)	2+ABX (mean 3.8 courses)	p-value
Number of gut bacteria	7.7 (SD 2.2)	5.1 (SD 2.3)	p=0.043
Number of gut fungi	0.78 (SD 0.83)	0.86 (SD 1.2)	p=0.88

Abbreviations: ABX: Antibiotic Courses in Past 12 Months

Table 6: Comparison of number of species isolated based on antibiotics received.

postulated that changes in the gut microbiome may even initiate the onset of malignant transformation in the GI tract [24]. As it has been established that functional endoscopic sinus surgery can lead to reduced courses of antibiotics postoperatively [25], perhaps preservation of the GI microbiome can serve as an additional reason to consider earlier surgical intervention in patients with CRS.

One drawback of the study relates to the storage of the stool samples. The investigators instructed patients to refrigerate the stool samples after collection at home. The patients may have variably adhered to these guidelines and the sample was unlikely to be refrigerated during transport to our facility. Gorzelak et al. [26] established that storage of samples at room temperature even beyond 15 min can reduce the diversity of extracted bacterial taxa. Another drawback of the study involves the nature of our patient set. Given that the patients were enrolled in a rhinologist's office, they tended to have diverse pathologies related to the sinuses but not related to the GI tract. None of the patients enrolled carried a known GI diagnosis. Given that some of the postulations regarding a link between the sinus and gut microbiome are based on patients with inflammatory bowel disease, it would improve the quality of similar study to include patients who carry these diagnoses.

Conclusion

Although there was minimal overlap between the sinus and gut microbiota in our study, further studies are needed with emphasis placed on patients with chronic diseases of the GI tract as well as in patients with CRS. Given that increasing numbers of antibiotic courses may lead to reduced diversity in the gut, preserving the gastrointestinal microbiome may be another reason to consider earlier surgical intervention in patients with chronic sinus disease.

Ethics Approval

Prior to commencement of this study, IRB ethics approval was obtained.

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