Gastrointestinal Tract

ORIGINAL ARTICLE

Small intestinal bacterial overgrowth is associated with laryngopharyngeal reflux symptom severity and impaired esophageal mucosal integrity

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ABSTRACT

Objectives: To explore the relationship between small intestinal bacterial overgrowth (SIBO) and laryngopharyngeal reflux (LPR). **Methods**: Data from patients undergoing high resolution manometry, pH-impedance monitoring, and hydrogen methane breath testing at a single tertiary center were analyzed retrospectively. SIBO was determined by a ≥ 20 ppm rise in breath hydrogen from baseline within 90 minutes after ingestion of lactulose. Patients were grouped by SIBO result and compared for subjective LPR symptoms on the Reflux Symptom Index (RSI) in addition to objective manometric and reflux parameters, including mean nocturnal baseline impedance (MNBI) in the proximal and distal esophagus. **Results**: Forty-one patients were analyzed, of which 46.3% were positive for SIBO. Patients with SIBO had a significantly greater LPR symptom burden on RSI (26.5 ± 9.3 *vs*. 16.9 ± 8.9, $P = 0.002$). Independently, SIBO was associated with throat clearing ($P = 0.016$), cough ($P < 0.001$) and globus ($P = 0.003$). Objectively, there was no difference in manometric or reflux parameters except patients with SIBO had significantly lower MNBI in the proximal esophagus (1970.2 ± 511.6 Ω *vs*. 2504.2 ± 816.1 Ω, $P = 0.026$). **Conclusion**: Patients with SIBO have greater LPR symptom severity and impaired mucosal integrity in the proximal esophagus. Future study should look to determine if treating SIBO improves symptoms of LPR.

Key words: laryngopharyngeal reflux, globus, baseline impedance, small intestinal bacterial overgrowth

INTRODUCTION

Laryngopharyngeal reflux (LPR) is an inflammatory condition of the upper aerodigestive tract tissues related to the direct and indirect effect of gastroduodenal content reflux, which induces morphological changes in the upper aerodigestive tract.[1] LPR symptom burden is commonly quantified by the reflux symptom index (RSI).[2] However, burden of LPR symptoms , including hoarseness, throat clearing, cough, globus, dysphagia, and dyspnea correlate poorly with reflux parameters on objective pH-impedance testing.[3] In addition, randomised controlled trials have demonstrated that proton pump inhibitors (PPIs) have no superiority to placebo for treating throat symptoms.[4,5] Therefore, raising doubt as to whether mucosal changes and laryngopharyngeal symptoms are related to gastric acid exposure. Another component of gastroduodenal reflux, pepsin, appears to distinguish patients with LPR from healthy controls[6] but has a relatively poor sensitivity and specificity for diagnosing LPR.[7] Moreover, when defining LPR by objective testing with multichannel

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intraluminal impedance, the presence of salivary pepsin correlates poorly with morbidity.[8]

Recently, an altered microbiota of the laryngopharynx has been proposed as a new pathophysiological paradigm for LPR symptoms and morphology.[9] There is increasing evidence that gram-negative bacteria are more abundant in the esophageal microbiome of patients with reflux related disease compared to controls.[10] However, the role of the intestinal microbiota in esophageal and oropharyngeal disorders is often overlooked despite it being implicated in many inflammatory diseases.[11] Small intestinal bacterial overgrowth (SIBO) is a condition related to the excessive colonisation of bacteria in the small bowel. Similarly to LPR, there is no widely accepted gold standard test for SIBO, but hydrogen and methane breath testing is cheap, non-invasive and recommended by societal guidelines.[12] SIBO is predominantly associated with symptoms of irritable bowel syndrome (IBS), but it was recently demonstrated that a large proportion of patients being worked up for gastroesophageal reflux disease (GERD) have SIBO.[13] Currently, the association between SIBO and LPR is unknown. In order to explore the hypothesis that SIBO is associated with LPR, we performed a retrospective review of patients referred for pH-impedance testing and hydrogen and methane breath testing at a single UK physiology center.

MATERIALS AND METHODS

Subjects and study design

This study retrospectively analyzed data from adult subjects who were referred for high resolution manometry (HRM), 24-hour pH-impedance monitoring, and hydrogen and methane breath testing as part of their standard care between January 2021 and June 2022. Exclusion criteria included previous gastrointestinal surgery, tests performed more than 10 weeks from each other, and incomplete tests. All patients consented for their data to be used anonymously in research.

High resolution manometry

HRM was performed using a solid-state catheter with 32 pressure sensors (Diversatek Healthcare, Milwaukee, WI) and analyzed according to Chicago Classification v4.0.[14] Ineffective esophageal motility (IEM) was defined by > 70% ineffective or ≥ 50% failed wet swallows. Hiatus hernia was determined on HRM by spatial separation of the lower esophageal sphincter (LES) and diaphragmatic crura pressure zones.

24-hour pH-impedance monitoring

24-hour ambulatory reflux monitoring was performed with a dual channel pH-impedance probe off PPI therapy. The probes consisted of either a gastric and esophageal pH sensor (ZAN-BG-44, Diversatek Healthcare) or esophageal and hypopharyngeal pH sensor (ZAI-BL-55 or ZAI-BL-56, Diversatek Healthcare) placed at 5cm above the LES or 1cm above the upper esophageal sphincter, respectively. Due to the heterogeneity of pH-impedance probes used in this cohort, hypopharyngeal events were not included in analysis. Mean nocturnal baseline impedance (MNBI) in the proximal esophagus was taken at Z1 with ZAN-BG-44 probes and Z3 with ZAI-BL-55/56 probes. MNBI in the distal esophagus was taken at Z5 from all probes. MNBI was generated as an average of three 10-minute resting periods during sleep. All patients were off PPIs for 7 days prior to pH-impedance testing.

Hydrogen and methane breath testing

Patients followed a low fermentable diet on the day prior to testing and performed the breath test after a 12-hour overnight fast. Hydrogen and methane breath testing was performed with 10 g lactulose substrate. A single baseline breath sample was provided and a further 9 breath samples every 15 minutes after ingestion of lactulose. A positive result for SIBO was defined as rise in breath hydrogen ≥ 20 ppm within 90 minutes after from baseline. Breath methane levels of ≥ 10 ppm at any interval during the test were considered positive for intestinal methanogen overgrowth (IMO). An inconclusive test was determined by a high baseline of ≥ 20 ppm or a flatline result where there was a rise of hydrogen < 10 ppm during the entire test.

Reflux symptom index

The RSI was completed on the day of pH-impedance testing. It is composed of 9 questions related to hoarseness, throat clearing, excess throat mucous or postnasal drip, dysphagia, coughing after eating or lying down, dyspnea, troublesome or annoying cough, globus and typical reflux symptoms (heartburn, chest pain or regurgitation). These are rated on a 0–5 Likert scale with 0 being no problem and 5 being severe problem. Based on response, patients were also split by symptom severity into none/mild (0–1), moderate (2–3), and severe (4–5).

Statistical analysis

Numerical data are reported as mean ± standard deviation unless otherwise stated. Categorical data are reported as frequencies and percentages unless otherwise stated. Distribution of data was determined by the Kolmogorov-Smirnov test. Numerical data between groups was compared with independent *t*-tests or Mann-Whitney tests for parametric and non-parametric data, respectively. Categorical data were compared using Pearson's chi quare test for association. The analysis was performed using JASP v0.16.0.

RESULTS

Data from 41 patients were analyzed and reasons for exclusion can be found in Figure 1. The mean age of the study population was 45.4 years (range 20–76 years), and 48.8% were female. Almost all patients (92.7%) reported at least one bothersome gas-related symptom including excessive belching (75.6%) and bloating (80.4%).

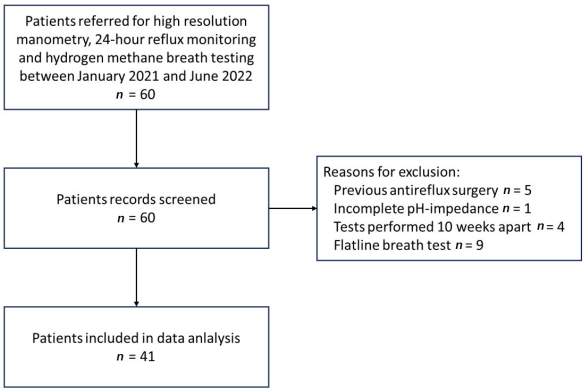

Figure 1. Flow diagram for patient selection process.

In total, 29 patients (70.7%) had intestinal dysbiosis on hydrogen and methane breath testing with 14 (34.1%) testing positive for SIBO only, 10 (24.4%) for IMO only, whilst 5 (12.2%) were positive for both SIBO and IMO (Table 1). Patients with SIBO had significantly greater RSI scores than those with IMO (27.2 ± 8.6 *vs.* 16.3 ± 11.4, $P = 0.049$) and all patients with SIBO vs those without SIBO (26.5 ± 9.3 *vs.*16.9 ± 8.9, $P = 0.002$). The mean RSI score was no different between those with and without IMO ($P > 0.05$). Independently, several symptoms on the RSI were associated with SIBO (Figure 2) including throat clearing ($P = 0.016$), coughing after meals or when lying down ($P = 0.019$), troublesome or annoying cough ($P < 0.001$) and globus ($P = 0.003$).

All patients were diagnosed with normal esophageal motility or ineffective esophageal motility on HRM, but this was not different based on breath test results ($P > 0.05$). Six patients had a ≥ 2 cm hiatus hernia and all tested negative for SIBO. For pH-impedance parameters, there was no difference between patients with and without SIBO or IMO, except patients with SIBO had a significantly lower MNBI in the proximal esophagus compared to those without SIBO (1970.2 ± 511.6 Ω *vs.* 2504.2 ± 816.1 Ω, $P = 0.026$). These data can be found in Table 2.

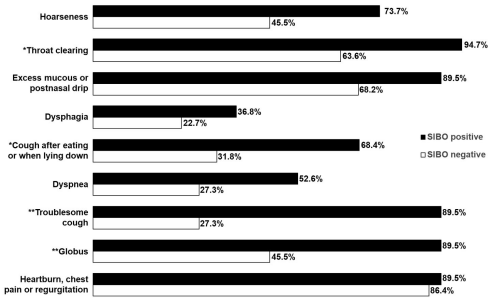

Figure 2. Frequency of moderate/severe symptoms reported on RSI in patients with and without SIBO. *$P < 0.05$. **$P < 0.01$.

DISCUSSION

To our knowledge, this is the first study to suggest a link between SIBO and LPR symptoms, with specific associations for throat clearing, cough, and globus pharyngeus. Objectively, acid exposure time and reflux event frequency were not different between groups, but MNBI in the proximal esophagus was significantly reduced in patients with SIBO. Kurylo and colleagues found that proximal MNBI was no different between patients with suspected LPR and healthy controls.[15] However, the mean proximal MNBI for patients in their study was similar to patients in our study who tested negative for SIBO. Baseline impedance is a marker of mucosal integrity and low baseline impedance is reflective of reflux burden.[16,17] Lower proximal esophageal impedance has also been shown to predict laryngopharyngeal symptoms and response to PPI therapy.[18,19] Therefore, low baseline impedance in the proximal esophagus may be related to long-term acid reflux exposure or other contents of gastroduodenal reflux that impair mucosal integrity.

The primary mechanism of gastroesophageal reflux is transient lower esophageal sphincter relaxations (TLESRs). There are two potential ways that SIBO may contribute to TLESRs. Firstly, TLESRs are induced by intestinal fermentation and distension.[20,21] Therefore, it could be suspected that patients with SIBO experience more TLESRs following ingestion of highly fermentable foods. Indeed, Plaidum and colleagues showed that compared to a meal low in fermentable carbohydrates (FODMAPs), a high FODMAP meal increased TLESRs and reflux symptom severity in patients with overlapping GERD and non-constipating IBS.[22] Conversely, the low FODMAP diet has been shown to reduce reflux symptom severity.[23] Since, these studies did not test for SIBO, it is uncertain whether subjects with SIBO were the ones to respond positively. Secondly, the bacteria chiefly responsible for SIBO are proteobacteria which produce lipopolysaccharide (LPS).[24] The LPS toxin has been shown to reduce LES basal tone and induce

Table 1: Demographic and clinical characteristics in patients with SIBO, IMO and both SIBO and IMO

	SIBO only (*n* = 14)	IMO only (*n* = 10)	SIBO and IMO (*n* = 5)	Negative (*n* = 12)
Age, years	40.9 ± 13.6	49.6 ± 16.4	58.6 ± 8.2	41.9 ± 14.0
Female	8 (57.1)	3 (30)	4 (80)	5 (41.7)
Manometric parameters				
UESP, mmHg	71.1 ± 88.3	64.8 ± 41.3	67.8 ± 73.7	106.4 ± 83.9
LESP, mmHg	20.3 ± 10.9	27.9 ± 15.1	32.0 ± 8.5	17.5 ± 9.4
Hiatus hernia, ≥ 2 cm	0 (0.0)[a]	2 (20.0)	0 (0.0)	4 (33.3)[a]
IEM	7 (50)	4 (40.0)	0 (0.0)	4 (33.3)
Reflux parameters				
AET ≥ 4%	6 (42.8)	2 (20.0)	2 (40.0)	3 (25.0)
Total AET, %	4.2 ± 4.9	2.9 ± 4.6	2.7 ± 2.8	2.8 ± 4.5
Total reflux	59.9 ± 28.6	37.6 ± 17.0	55.4 ± 23.4	67.3 ± 82.9
Proximal reflux	30.1 ± 20.6	18.7 ± 14.7	22.0 ± 19.6	30.5 ± 48.2
Distal MNBI, Ω	2182.6 ± 1326.6	2284.7 ± 1162.4	2349.2 ± 499.7	2871.8 ± 1048.4
Proximal MNBI, Ω	1958.1 ± 499.8[a]	2303.2 ± 911.6	1999.2 ± 599.0	2685.9 ± 718.5[a]
Symptom index parameters				
Positive SI/SAP	7 (53.8)	1 (12.5)	4 (100.0)	6 (50)
RSI	27.2 ± 8.6[a,b]	16.3 ± 11.4[a]	24.6 ± 12.0	17.5 ± 6.6[a,b]

Note: All values expressed as mean ± standard deviation or frequencies and percentages. [a,b]$P < 0.05$ between groups. UESP: upper esophageal sphincter pressure (normal range 30–118 mmHg); LESP: lower esophageal sphincter pressure (normal range 10–45 mmHg), IEM: ineffective esophageal motility; AET: acid exposure time (normal range < 4%); MNBI: mean nocturnal baseline impedance; SI/SAP: symptom index/symptom association probability; RSI: reflux symptom index; SIBO: small intestinal bacterial overgrowth; IMO: intestinal methanogen overgrowth

Table 2: Demographic and clinical characteristics in patients with and without SIBO

	SIBO positive (*n* = 19)	SIBO negative (*n* = 22)	*P* value
Age, years	45.5 ± 14.6	45.4 ± 15.3	0.980
Female	12 (63.2)	8 (36.4)	0.087
Manometric parameters			
UESP, mmHg	70.2 ± 82.5	88.6 ± 70.7	0.073
LESP, mmHg	23.4 ± 11.4	22.3 ± 13.2	0.625
Hiatus hernia, ≥ 2 cm	0 (0.0)	6 (27.2)	0.014
IEM	7 (36.8)	8 (36.4)	0.975
Reflux parameters			
AET ≥ 4%	8 (42.1)	5 (22.7)	0.184
Total AET, %	3.8 ± 4.4	2.8 ± 4.4	0.283
Total reflux	58.7 ± 26.8	53.8 ± 62.9	0.082
Proximal reflux	27.9 ± 20.1	25.4 ± 37.4	0.212
Distal MNBI, Ω	2226.4 ± 1154.2	2604.9 ± 1115.5	0.293
Proximal MNBI, Ω	1970.2 ± 511.6	2504.6 ± 816.1	0.033
Symptom index parameters			
Positive SI/SAP	11 (64.7)	7 (35.0)	0.072
RSI	26.5 ± 9.3	16.9 ± 8.9	0.002

Note: All values expressed as mean ± standard deviation or frequencies and percentages. UESP: upper esophageal sphincter pressure (normal range 30–118 mmHg); LESP: lower esophageal sphincter pressure (normal range 10–45 mmHg); IEM: ineffective esophageal motility; AET: acid exposure time (normal range < 4%); MNBI: mean nocturnal baseline impedance; SI/SAP: symptom index/symptom association probability; RSI: reflux symptom index; SIBO: small intestinal bacterial overgrowth

TLESRs.[25]

Unfortunately, due to the retrospective nature of the study, we were unable to follow up with all patients who tested positive for SIBO to see if LPR symptoms improved following SIBO eradication. Interestingly, the most common treatment for SIBO, rifaximin, has been shown to improve dyspeptic symptoms, even in patients without SIBO, which may be attributed to anti-inflammatory effects and modulation of visceral hypersensitivity.[26] Another limitation of this study is the small sample size, especially since we removed 15% of the initial cohort due to an inconclusive breath test, mostly related to a flatline result. This is because a flatline breath test is abnormal since a rise in breath hydrogen is expected when lactulose arrives at the cecum, and so a flatline result may be suggestive of delayed oro-cecal transit time or the presence of hydrogenotrophic bacteria (*i.e.*, hydrogen sulfide-producing bacteria).[27]

Due to the retrospective nature of the study, different pH-impedance probes were used in the patients and therefore we were unable to determine the differences in hypopharyngeal reflux events between groups. Also, some of the subjects were not reviewed by nasoendoscopy. Whilst this is useful to rule out structural or neoplastic pathology, the only validated observer-based endoscopic scoring system for LPR, the Reflux Finding Score (RFS), has not been found to correlate with objective measures of LPR on pH-impedance,[28] so this is probably not a major omission. Hypopharyngeal pH-impedance monitoring has recently been proposed as the "gold standard" test for LPR where more than one hypopharyngeal reflux event is abnormal.[29] Therefore, we were unable to objectively quantify LPR.

Finally, our cohort of patients were referred for reflux monitoring and breath testing as part of their standard care. Most of the patients reported at least one troublesome gas-related symptom of belching or bloating, which may explain why they were referred for hydrogen and methane breath testing to exclude intestinal dysbiosis as a cause of gaseous symptoms. We were unable to determine the prevalence of functional gastrointestinal disorders due to the absence of ROME questionnaires. However, we previously showed that SIBO and IMO are common in patients being worked up for GERD.[13] In particular, we found that SIBO was associated with a positive reflux symptom association *i.e.*, patient symptoms are more likely to be related to reflux.[13] There was also a trend towards these findings in this separate cohort as seen in Table 2.

CONCLUSION

Patients who test positive for SIBO exhibit higher self-reported laryngopharyngeal symptom scores than those who test negative. Objective impedance testing shows that patients with SIBO have signs of impaired mucosal integrity in the proximal esophagus. Further studies should elucidate whether treating SIBO improves symptoms of LPR. We suggest that hydrogen and methane breath testing for SIBO could be utilized as an adjunctive test alongside hypopharyngeal pH-impedance monitoring in those with suspected LPR.

DECLARATIONS

Author contributions

Haworth JJ and Otterstad M extracted data. Haworth JJ and Otterstad M analyzed data. Haworth JJ wrote first draft of manuscript. Hobson AR and Birchall MA revised manuscript.

Informed consent statement

Written informed consent was obtained from the patients for their anonymized information to be published in this manuscript.

Conflict of interest

All authors have no conflict of interest related to the manuscript.

Data sharing statement

The original anonymous dataset is available on request from the corresponding author at jordan@functionalgutdiagnostics.com.

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