

## Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome: A Bridge between Functional Organic Dichotomy

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The pathogenesis of irritable bowel syndrome (IBS), once thought to be largely psychogenic in origin, is now understood to be multifactorial. One of the reasons for this paradigm shift is the realization that gut dysbiosis, including small intestinal bacterial overgrowth (SIBO), causes IBS symptoms. Between 4% and 78% of patients with IBS and 1% and 40% of controls have SIBO; such wide variations in prevalence might result from population differences, IBS diagnostic criteria, and, most importantly, methods to diagnose SIBO. Although quantitative jejunal aspirate culture is considered the gold standard for the diagnosis of SIBO, noninvasive hydrogen breath tests have been popular. Although the glucose hydrogen breath test is highly specific, its sensitivity is low; in contrast, the early-peak criteria in the lactulose hydrogen breath test are highly nonspecific. Female gender, older age, diarrhea-predominant IBS, bloating and flatulence, proton pump inhibitor and narcotic intake, and low hemoglobin are associated with SIBO among IBS patients. Several therapeutic trials targeting gut microbes using antibiotics and probiotics have further demonstrated that not all symptoms in patients with IBS originate in the brain but rather in the gut, providing support for the micro-organic basis of IBS. A recent proof-of-concept study showing the high frequency of symptom improvement in patients with IBS with SIBO further supports this hypothesis. (**Gut Liver 2017;11:196-208**)

**Key Words:** Bacterial overgrowth; Dysbiosis; Breath tests; Gastrointestinal microbiota; Probiotics; Rifaximin

### INTRODUCTION

Irritable bowel syndrome (IBS) is one of the commonest disorders encountered in Gastroenterology practice.<sup>1</sup> IBS is manifested by abdominal pain and/or discomfort, irregular stool

form and passage.<sup>2</sup> Bloating is another common symptom of IBS.<sup>2,3</sup> Patients with small intestinal bacterial overgrowth (SIBO), in which there is increase in bacteria equal to or greater than  $10^5$  colony forming unit per mL of upper gut aspirate,<sup>2</sup> also experience abdominal pain or discomfort, bloating, flatulence and loose motion.<sup>4,5</sup> In contrast to the earlier belief, SIBO is known to occur in absence of anatomical factors predisposing to it.<sup>6</sup> A proportion of patients with IBS are known to have SIBO.<sup>7</sup> Recent realization that SIBO may be associated with symptoms of IBS, led to a paradigm shift in understanding the pathogenesis of this condition, hitherto thought to be related largely to psychological factors,<sup>8</sup> to more organic nature.<sup>7</sup> Such realization is well known in other conditions as well; for example, peptic ulcer, once thought to be related to psychological stress,<sup>9</sup> is now known to be related to infection with *Helicobacter pylori*.<sup>10,11</sup> Hence, it is worthwhile reviewing the existing literature on SIBO and IBS.

### GUT FLORA AND SIBO

Human gut harbor  $10^{14}$  bacterial cells, which are 10 times higher than the number of cells in the human body.<sup>12</sup> Gastrointestinal (GI) tract is considered as the most heavily colonized organ and more than 70% of microbes reside in colon.<sup>13</sup> The human GI tract is inhabited by a vast number of microbial population including bacteria, fungi, and viruses.<sup>14</sup> Bacteria contribute to the largest population of gut microbiota, consisting of 500 (using culture approaches) to 1,000 (by 16S rRNA gene sequencing) different bacterial species.<sup>15</sup> The number of bacteria increases from stomach ( $10^1$  to  $10^3$  bacteria/g) to the colon ( $10^{11}$  to  $10^{12}$  bacteria/g).<sup>13</sup> The small intestine comprises mainly of Gram positive and aerobic bacteria and the large intestine contains predominantly Gram negative and anaerobic bacteria.<sup>16</sup> Majority of bacteria residing in the colon are strictly

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anaerobes (95% of total) followed by facultative anaerobes and aerobes.<sup>15</sup> More than 50 bacterial phyla have been identified in human gut.<sup>17</sup> Major phyla residing in the gut are Bacteroidetes and Firmicutes, whilst Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria are present in minor proportion.<sup>13,18</sup>

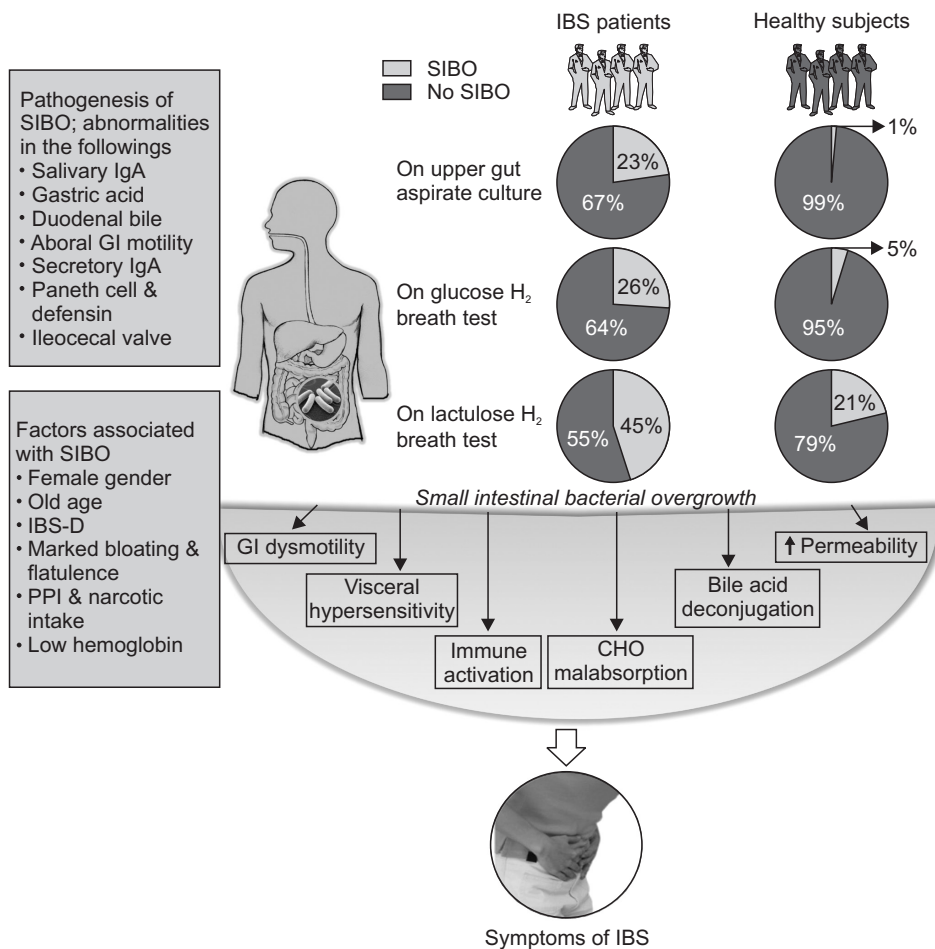
Normal gut flora may provide several beneficial effects to the host. These include fermentation of un-digested dietary residue and endogenous mucus producing short chain fatty acids, which are nutrients to the colonic epithelial cells and conservation of energy, absorption of NaCl and water, particularly from the right colon, synthesis of vitamin K, control of epithelial cell proliferation, protection against pathogens by a barrier effect and training of the immune system.<sup>19-21</sup> One study showed that small intestine of germ free animal has thin and irregular villi, reduced crypt size, increased number of Peyer's patches, and infiltration of leukocytes in lamina propria.<sup>22,23</sup> Alteration in the normal flora leads to disturbance in the intestinal homeostasis.<sup>2</sup> There are several intrinsic and extrinsic factors that prevent overgrowth of bacteria in the small intestine. Intrinsic factors include: (1) secretion of gastric juice and bile, which have antibacterial effect; (2) peristaltic movement preventing adherence of bacteria into the intestinal mucosa; (3) normal gut defense including humoral and cellular mechanisms; (4) mucin production by intestinal mucosal epithelial cell inhibiting pathogenic bacteria; (5) gut antibacterial peptides such as defensins; and (6) ileocecal valve preventing retrograde translocation of bacteria from colon to the small intestine.<sup>24-26</sup> Extrinsic factors include diet and drugs modulating gut flora, such as pre and probiotics, gastric acid suppressants such as proton pump inhibitors (PPIs), H<sub>2</sub> blockers, and antibiotics and drugs altering motility (prokinetics, anticholinergics, and opioids).<sup>4,22,27-30</sup> If, there is failure of any of the above-mentioned protective mechanisms, it may lead to development of SIBO (Fig. 1).

Though quantitative culture of the upper gut aspirate has traditionally been used as the gold standard for the diagnosis SIBO, its limitations include difficulty and invasiveness, cost, contamination by oropharyngeal flora, and inability to culture as high as 70% bacteria colonizing the gut.<sup>2,13,30,31</sup> Moreover, distribution of bacterial overgrowth may be patchy and upper gut aspirate may not be able to detect bacterial overgrowth in distal gut.<sup>30,32</sup> The anaerobic bacteria may not grow if air is used during endoscopy; hence, either nitrogen or carbon dioxide is better for this purpose. In one of our earlier studies in which we used air during endoscopy, of 34 of 50 patients with malabsorption syndrome in whom bacteria were cultured in jejunal aspirate, only one grew anaerobic bacteria.<sup>33</sup> Hence, search for other less invasive and patient-friendly methods for diagnosis of SIBO continues.

In an attempt to overcome some of the limitations of the traditional culture-based method for diagnosis of SIBO, a novel technology, called culturomics, has been developed recently.<sup>15</sup>

Culturomics confer a new platform for identification of large number of bacterial colonies as well as noncultivable species in a short time duration using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF).<sup>15,34</sup> In a recent study, using 212 different culture conditions, 340 different bacterial, 5 fungal species and one virus were identified, including 31 new species using culturomics (MALDI-TOF) technique.<sup>34,35</sup> Thus, culturomic approaches are feasible, rapid, cost-effective and reproducible for the study of gut microbiota.<sup>15,34</sup> However, studies on SIBO using culturomics method are lacking. Moreover, use of effective culture conditions and sequencing methods may make it rarely usable for routine clinical application.

Breath tests are popular, noninvasive and patient-friendly methods used increasingly for diagnosis of SIBO.<sup>36</sup> Diagnostic role of hydrogen breath tests depends on the type of the substrates used; for example, lactose and fructose hydrogen breath tests are useful for carbohydrate malabsorption; on the other hand, glucose and lactulose hydrogen breath tests (GHBT and LHBT) are useful for diagnosis of SIBO, the former being more specific. Therefore, choice of the substrate while performing hydrogen breath test is important as only specific substrate diagnoses SIBO and others test for carbohydrate malabsorption.<sup>36</sup> Hydrogen and methane gases are produced by the gut flora from the ingested substrates, particularly the colonic flora in patients with carbohydrate malabsorption and from small bowel bacteria in patients with SIBO.<sup>7,24,37</sup> Eighty percent of the gases like hydrogen and methane are eliminated with the flatus and the remaining 20% are absorbed and exhaled by lung, which can be measured in breath.<sup>22,38</sup> In GHBT, rise in hydrogen by 12 parts per million (ppm) above basal following administration of 50 to 100 g glucose due to bacterial fermentation of the substrate in small intestine is diagnostic of SIBO.<sup>36</sup> A recent study showed that measuring methane does not increase the yield of hydrogen breath test to diagnose SIBO.<sup>39</sup> In presence of SIBO, two peaks may be seen during LHBT: the first one due to bacterial fermentation of lactulose in small bowel and the second one after lactulose reaches colon.<sup>36</sup> Since number of bacteria in colon is higher than that in the small bowel even in patients with SIBO, a rise in breath hydrogen more than 20 ppm above basal is expected from colonic fermentation of the lactulose.<sup>40</sup> Though GHBT is highly specific (78% to 97%),<sup>41,42</sup> it is quite insensitive (15.7% to 62%).<sup>42,43</sup> In contrast, conventional double-peak criteria on LHBT lack sensitivity (31% to 68%) and the recently proposed early-peak criterion (rise in breath hydrogen within 90 minutes by 20 ppm above basal following lactulose ingestion) often gives false positive result with specificity of 65% to 97.9%.<sup>39,44</sup> This is the reason for overestimation of frequency SIBO (as high as 78%) in the initial studies from United States.<sup>40</sup> In fact, the early-peak criterion on LHBT, which was used in the initial studies on SIBO in patients with IBS, presumed that normal mouth to cecum transit time is more than 90 minutes in spite of the observation that it may be shorter.<sup>36,40</sup> A study that



**Fig. 1.** Schematic diagram showing the frequency of small intestinal bacterial overgrowth (SIBO) using quantitative jejunal aspirate culture, glucose and lactulose hydrogen breath tests (GHBT and LHBT, respectively) among patients with irritable bowel syndrome (IBS), gut defense mechanisms that prevent the development of SIBO, factors associated with SIBO among patients with IBS, and mechanisms of IBS symptom development. As shown in the figure, the frequency of SIBO in IBS patients using LHBT (early-peak criteria) is higher than that by using upper gut aspirate culture and GHBT (LHBT [45%]; upper gut aspirate culture [23%] and GHBT [26%]). Moreover, SIBO is more frequent in healthy controls using LHBT due to false positive test results (LHBT [21%], upper gut aspirate culture [1%] and GHBT [5%]). GI, gastrointestinal; IBS-D, irritable bowel syndrome, diarrhea-predominant; PPI, proton pump inhibitor; CHO, carbohydrate.

combined radio-nuclide gut transit and LHBT revealed that in most patients in whom a peak in hydrogen was seen on LHBT, radio-nuclide already arrived in cecum.<sup>45</sup> Other methods for diagnosis of SIBO include CO<sub>2</sub> breath tests (<sup>14</sup>C or <sup>13</sup>C D-xylose, <sup>13</sup>C glucose and <sup>13</sup>C cholyl-glycine hydrolase).<sup>7,22,46,47</sup>

Though hydrogen breath tests are quite popular for the diagnosis of SIBO, these are not free from limitations. In patients with distal SIBO, GHBT may be falsely negative as glucose gets completely absorbed in the proximal small bowel and hence, may not reach the site of SIBO.<sup>5,31,36</sup> In patients with fast gut transit, early peak criteria proposed by Pimentel *et al.*<sup>40</sup> often give false positive results.<sup>36</sup> Fast gut transit is not uncommon, particularly in Asia. In a study from our center, median oro-cecal transit time in healthy subjects was 65 minutes (range, 40 to 110 minutes).<sup>48</sup> A Taiwanese study revealed that average oro-cecal transit time was 85±37 minutes.<sup>49</sup> Hence, it is important to realize that there is need to search for a noninvasive yet sensitive and specific method for diagnosis of SIBO.

**FREQUENCY OF SIBO IN PATIENTS WITH IBS**

There are several studies evaluating frequency of SIBO among patients with IBS as compared with controls using different di-

agnostic methods such as GHBT, LHBT and quantitative upper gut aspirate culture.<sup>7</sup> Table 1 summarizes the results of these studies. As shown in the Table 1, frequency SIBO among patients with IBS ranged between 4% and 78% and that among controls, between 1% and 40%.<sup>37,39,40,45,50-68</sup> Most case-control studies revealed that SIBO was commoner among IBS than controls; this suggests that there is significant association between SIBO and IBS (Fig. 1).

Two meta-analyses also suggested association between IBS and SIBO. In a meta-analysis by Ford *et al.*,<sup>69</sup> of the 12 studies including 1,921 patients with IBS, pooled prevalence of a positive LHBT and GHBT was 54% (95% confidence interval [CI], 32% to 76%) and 31% (95% CI, 14% to 50%), respectively. The odds ratio (OR) for any test showing positive SIBO result among patients with IBS as compared to controls was 3.45 to 4.7.<sup>69</sup> In another meta-analysis on 11 studies, breath testing was found to be abnormal among patients with IBS than controls (OR, 4.46; 95% CI, 1.69 to 11.80). Breath testing had an overall sensitivity and specificity in separating IBS patients from healthy subjects of 44% and 84%, respectively.<sup>42</sup> However, in this meta-analysis, authors suggested that abnormal breath test might not always mean SIBO as rise in breath hydrogen greater than 20 ppm above basal has poor specificity to diagnose SIBO.<sup>42</sup>

**Table 1.** Prevalence of Small Intestinal Bacterial Overgrowth among Patients with Irritable Bowel Syndrome

Study no.	Prevalence of SIBO in cases	Prevalence of SIBO in controls	Methane producers in cases	Methane producers in controls	Country	Year	Reference
Culture of jejunal aspirate ( $\geq 10^5$ CFU/mL colonic-type bacteria)							
1	7/162 (4)	1/26 (4)	ND	ND	Sweden	2007	Posserud <i>et al.</i> <sup>52</sup>
2	4/12 (33)	0/9	ND	ND	The Netherlands	2008	Kerckhoffs <i>et al.</i> <sup>53</sup>
3	15/80 (18)	0/10	2/15 (13)	ND	India	2014	Ghoshal <i>et al.</i> <sup>39</sup>
4	42/112 (37)	ND	ND	ND	Greece	2012	Pyleris <i>et al.</i> <sup>54</sup>
Culture of jejunal aspirate ( $\geq 10^3$ CFU/mL colonic-type bacteria)							
5	62/139 (44.6)	ND	ND	ND	United States	2015	Erdogan <i>et al.</i> <sup>27</sup>
Lactulose hydrogen breath test							
6	157/202 (78)	ND	ND	ND	United States	2000	Pimentel <i>et al.</i> <sup>40</sup>
7	64/98 (65)	ND	ND	ND	Italy	2005	Nucera <i>et al.</i> <sup>37</sup>
8	39/390 (10)	ND	ND	ND	Canada	2005	Walters and Vanner <sup>55</sup>
9	35/89 (39)	1/13 (8)	ND	ND	China	2014	Zhao <i>et al.</i> <sup>56</sup>
10	25/40 (63)	ND	ND	ND	Canada	2011	Yu <i>et al.</i> <sup>45</sup>
11	34/76 (45)	16/40 (40)	19/76 (25)	10/40 (25)	Korea	2010	Park <i>et al.</i> <sup>57</sup>
12	28/43 (65)	4/56 (7)	4/43 (9)	0	Italy	2009	Scarpellini <i>et al.</i> <sup>58</sup>
13	55/127 (43)	ND	ND	ND	Italy	2008	Carrara <i>et al.</i> <sup>59</sup>
14	89/258 (34.5)	ND	ND	ND	United States	2009	Mann and Limoges-Gonzales <sup>60</sup>
15	60/175 (34.3)	45/150 (30)	ND	ND	India	2008	Rana <i>et al.</i> <sup>61</sup>
16	22/119 (18.4)	ND	ND	ND	Pakistan	2011	Yakoob <i>et al.</i> <sup>62</sup>
Glucose hydrogen breath test							
17	25/225 (11.1)	1/100 (1)	ND	ND	India	2012	Rana <i>et al.</i> <sup>67</sup>
18	93/204 (46)	ND	27/204 (13)	ND	United States	2007	Majewski and McCallum <sup>63</sup>
19	105/331 (32)	7/105 (7)	ND	ND	Rome	2014	Moraru <i>et al.</i> <sup>70</sup>
20	14/59 (24)	1/37 (2.7)	5/59 (9)	9/37 (24)	India	2011	Sachdeva <i>et al.</i> <sup>64</sup>
21	11/129 (8.5)	1/51 (2)	ND	ND	India	2010	Ghoshal <i>et al.</i> <sup>51</sup>
22	44/96 (45.8)	ND	ND	ND	Italy	2006	Cuoco and Salvagnini <sup>65</sup>
23	20/65 (31)	4/102 (4)	ND	ND	Italy	2005	Lupascu <i>et al.</i> <sup>66</sup>
24	8/72 (11.1)	ND	ND	ND	Rome	2013	Moraru <i>et al.</i> <sup>50</sup>
25	49/200 (24.5)	3/50 (6)	ND	ND	Italy	2010	Lombardo <i>et al.</i> <sup>143</sup>
26	38/139 (27.3)	ND	ND	ND	United States	2015	Erdogan <i>et al.</i> <sup>27</sup>
27	11/175 (6.2)	1/150 (0.66)	ND	ND	India	2012	Rana <i>et al.</i> <sup>97</sup>
28	40/107 (37.3)	14/107 (13)	ND	ND	Iran	2015	Abbasi <i>et al.</i> <sup>68</sup>

Data are presented as number (%).

SIBO, small intestinal bacterial overgrowth; CFU, colony forming unit; ND, not done.

In last half a decade, after these meta-analyses were published, at the time of writing this review, about eight case-control studies on frequency of SIBO among patients with IBS have been published (Table 1). Two of these studies were published from United States, three from India, one each from Pakistan, Romania, and Iran.<sup>27,39,54,62,64,67,68,70</sup> Of the five studies, which compared the frequency of SIBO among patients with IBS as compared to healthy controls, four showed significant difference. In these studies, frequency of SIBO among patients with IBS ranged between 19% and 37% and that among healthy controls between 0% and 12%.<sup>27,39,54,62,64,67,68,70</sup> It is worthwhile

evaluating the reasons for such wide variation in frequency of SIBO in these studies.

#### EXPLANATION FOR DIFFERENCE IN PREVALENCE OF SIBO IN IBS

Variations in prevalence of SIBO in patients with IBS and controls in several studies might be attributed to difference in geographical origin of studied population, different criteria for diagnosis of IBS (such as Manning, Rome I, II, and III), and methods for diagnosis of SIBO using different breath tests (such

as nature of substrates, gases analyzed, instrument).<sup>51</sup> Early peak criteria of LHBT give higher frequency of SIBO among patients with IBS and controls than other diagnostic methods, which might be attributed to false positive results.<sup>7,40,57,59,60</sup> Whilst, double peak criteria on LHBT and GHBT give low frequency of SIBO in IBS patients and controls, which might be due to low sensitivity.<sup>7</sup> Advanced noninvasive diagnostic techniques must be standardized for therapeutic management of SIBO in patients with IBS.

### FACTORS ASSOCIATED WITH SIBO AMONG PATIENTS WITH IBS

There are several factors that are associated with SIBO among patients with IBS. These include female gender, older age, predominant symptom of bloating and flatulence, and diarrheal subtype of IBS.<sup>4,71</sup> In fact, in a recent study, we found that number of bacterial colonies in the small bowel influenced Bristol stool type with higher number being associated with looser stools.<sup>39</sup> Similar observation has been reproduced in another recent study.<sup>72</sup> Since many patients with IBS might be taking PPIs due to overlapping dyspepsia, and PPI intake may influence development of SIBO, this may be one factor predicting occurrence of SIBO among patients with IBS.<sup>4,73</sup> Narcotic intake might be another factor causing SIBO among patients with IBS due to slowing of gut motility.<sup>74</sup> In one study, we found that lower value hemoglobin was associated with SIBO on GHBT.<sup>51</sup> Subjects with older age are more susceptible to SIBO, most likely as a result of reduced GI motility, intestinal surgery, small bowel diverticulosis and use of medications.<sup>75</sup> One previous study reported that patients older than 55 years with symptoms of IBS, particularly abdominal bloating and flatulence were more likely to be positive by GHBT.<sup>4</sup> Abdominal bloating among patients with IBS might be due to excess gas production by bacterial fermentation of undigested carbohydrates, in addition to SIBO.<sup>76</sup>

### MICROBIOLOGICAL ASPECTS OF SIBO IN IBS

SIBO can be classified into two categories based on difference in bacterial flora: (1) Gram positive flora might be due to failure of gastric acid barrier, and (2) coliform bacteria might be due to failure of intestinal clearance and small bowel anatomical alterations.<sup>52,77</sup> Recently, one study based on culture of jejunal aspirates showed that *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter lwoffii*, *Staphylococcus species*, *Klebsiella pneumoniae*, *Streptococcus species*, *Acinetobacter baumannii*, *Enterococcus faecalis*, and *Enterococcus faecium* were dominant bacteria among patients with SIBO.<sup>39</sup> Pyleris *et al.*<sup>54</sup> reported that of 42/112 patients with IBS having SIBO, *E. coli*, *Enterococcus species* and *K. pneumoniae* were the predominant species. Gram negative bacilli and *Enterobacter* were most common on culture of jejunal aspirates among patients with IBS.<sup>54</sup>

### PATHOGENESIS OF IBS SYMPTOMS AMONG PATIENTS WITH SIBO

Though the pathophysiology of IBS remains largely enigmatic, evidence from recent studies does show that dysbiosis may contribute to development of symptoms, at least in a subset of patients.<sup>2,78</sup> Though SIBO is a form of quantitative alteration of small bowel microbes, altered microbiota (dysbiosis) does not necessarily mean SIBO only. Dysbiosis includes qualitative alteration of gut flora (most authors reported on fecal microbiota) but also its quantitative change (SIBO).<sup>79,80</sup> In the recent Rome IV review, importance of dysbiosis including that of SIBO has been recognized by several experts.<sup>81-83</sup> In SIBO, bacterial fermentation of diet in the lumen produces hydrogen, methane and carbon dioxide gases, which may contribute to symptoms like distension, flatulence, abdominal pain, and bloating.<sup>2,22</sup> Methane is known to slow gut transit resulting in constipation.<sup>84</sup> These gases, however, may also be produced in the colon among patients without SIBO in presence of carbohydrate malabsorption.

SIBO is more often associated with diarrhea than constipation-predominant IBS.<sup>7</sup> Mechanism of diarrhea in patients with SIBO include de-conjugation of bile salts, enterotoxic effect of bacterial metabolites, increased small intestinal permeability, deficiency of vitamin B<sub>12</sub> and low grade inflammation resulting from immune activation in the small intestinal mucosa.<sup>22,37,85</sup>

Secondary deficiency of disaccharidases (e.g., lactase) is well known in patients with SIBO.<sup>86,87</sup> This results in maldigestion of carbohydrates such as lactulose, sucrose and sorbitol.<sup>86,87</sup> Moreover, fermentation of carbohydrates leads to formation of short chain fatty acids like acetic acid, propionic acid and butyric acid.<sup>88</sup> Though short chain fatty acids are useful for colon by providing nutrients to the colonocytes, conservation of energy and absorption of water and electrolytes, in the small bowel, it inhibits nutrient absorption and inhibits jejunal motility (ileal brake) through liberation of peptide YY, neurotensin and glucagon like peptide-1, which promotes SIBO.<sup>89</sup> Lipopolysaccharides derived from Gram negative bacteria may also affect the GI motility.<sup>90</sup> Furthermore, bacterial derived metabolites may affect colonic motility. Likewise, formyl-methionyl-leucyl-phenylalanine may affect the enteric nervous system.<sup>91-94</sup>

Bacteria utilize intraluminal proteins leading to production of ammonia.<sup>95</sup> Bacterial overgrowth produces a number of toxic compounds (peptidoglycans, D-lactate and serum amyloid A), which promote inflammation, may damage the brush border of the enterocytes and increases small intestinal permeability.<sup>22,95</sup> Host response to SIBO also depends on its genetic make-up as evidenced by a recent case-control study on 209 patients with IBS and 273 healthy subjects.<sup>96</sup> Under-producer genotypes of interleukin 1 (IL-1) receptor antagonist gene (anti-inflammatory) were associated with IBS.<sup>96</sup> Moreover, IBS patients had higher levels of IL-1  $\alpha$  and  $\beta$  than those without SIBO.<sup>96</sup> Another study showed higher level of proinflammatory cytokines such as IL-6



and tumor necrosis factor  $\alpha$  among patients with IBS-D than controls.<sup>97</sup> In addition, SIBO is associated with increased level of serum endotoxins, inflammatory cytokines and chemokines, and endogenous production of ethanol.<sup>23,95,98</sup>

Increased number of enterochromaffin cells was found in the mucosa of colon and rectum among patients with IBS than healthy controls.<sup>99</sup> Immune activation in response to SIBO recruits increased number of intraepithelial lymphocytes, mast cells and enterochromaffin cells.<sup>100</sup> Moreover, mediators of host immune response trigger the enteric nervous system altering GI motility and visceral hypersensitivity, which are the major pathophysiological mechanisms of IBS.<sup>101</sup>

Overgrowth of sulfate reducing bacteria may play an important role in patients with IBS.<sup>102</sup> An association was found between bacterial derived hydrogen sulfide ( $H_2S$ ) and visceral hypersensitivity.<sup>103</sup>  $H_2S$  is known to act as gaseous neurotransmitters inducing the contraction of detrusor muscle in the urinary bladder.<sup>103</sup> Recently, a study has shown that  $H_2S$  produced by sulphate reducing bacteria may play role in pathogenesis of SIBO. The author suggested that breath  $H_2S$  could be considered as potential noninvasive biomarker for diagnosis of SIBO among patients with IBS-D.<sup>104</sup>

Fibromyalgia, a condition associated with IBS, is also associated with SIBO.<sup>99</sup> A study by Pimental *et al.*<sup>105</sup> showed that all 42 patients with fibromyalgia had positive LHBST. This percentage was significantly higher than the control population (3/15, 20%). These data might suggest that somatic hypersensitivity is also influenced by altered gut flora.

## TREATMENT OF SIBO IN PATIENTS WITH IBS

There are several approaches to treat SIBO among patients with IBS that include antibiotics, probiotics and prokinetics.<sup>106</sup> Dietary manipulation has potential influence on the gut microbiota that may relieve some of the symptoms of SIBO.<sup>2,22,107</sup> Recently, utility of therapeutic manipulation of gut flora using antibiotic and probiotic<sup>2</sup> to treat IBS is being increasingly recognized and hence, worth reviewing.

### 1. Antibiotics

While choosing antibiotics, one should consider whether its antibacterial spectrum is broad including aerobes and anaerobes and absorption is poor reducing systemic side effects.<sup>108</sup> Though in the past, tetracycline, doxycycline, co-trimoxazole, fluoroquinolones have all been used in the treatment of SIBO,<sup>109,110</sup> in most of the recent studies among patients with IBS, rifaximin has been the preferred antibiotic (Table 2).<sup>52,108,111-114</sup>

Rifaximin is a semi-synthetic, nonabsorbable antimicrobial agent that acts against Gram positive and Gram negative aerobic and anaerobic bacteria.<sup>108</sup> Pimental *et al.*<sup>115</sup> reported two identically designed, large, multicenter, double blind, placebo-controlled trials (TARGET 1 and TARGET 2) among patients

**Table 2.** Clinical Trials of Antibiotics among Patients with Small Intestinal Bacterial Overgrowth and Irritable Bowel Syndrome

Study no.	Antibiotics (dosage)	Duration, day	Study subject	Clinical outcome	Reference
1	Rifaximin (1,600 mg/day) vs rifaximin (1,200 mg/day)	7	80 Patients with SIBO	Rate of normalization of GHBT was greater with higher dose of rifaximin than lower dose (80% vs 58%, p<0.05).	Scarpellini <i>et al.</i> <sup>108</sup>
2	Rifaximin (group 1, 600; group 2, 800; group 3, 1,200 mg/day)	7	90 Patients with SIBO and 30 patients in each group	Rate of normalization of GHBT was higher in group 3 than group 1 and 2 (60% vs 17%, p<0.001; 60% vs 27%, p<0.01).	Lauritano <i>et al.</i> <sup>112</sup>
3	Neomycin (n=55) or placebo (n=56)	7	111 Patients with IBS	Neomycin reduced the symptoms of IBS more often than placebo (35% vs 11%, p<0.05) and normalized lactulose hydrogen breath test result.	Pimental <i>et al.</i> <sup>113</sup>
4	Ciprofloxacin (500 mg, twice daily)	10	7 Patients with SIBO	Ciprofloxacin decreased viable bacterial counts in five patients (71%), while four (57%) still fulfilled criteria for SIBO. Three patients (43%) reported at least 25% improvement in IBS symptoms.	Posserud <i>et al.</i> <sup>52</sup>
5	Norfloxacin (800 mg/day) or placebo	10	80 IBS patients	Norfloxacin significantly reduced the symptom scores among patients with SIBO than without but not with placebo at 1 month. Symptoms resolved to turn Rome III negative more often in SIBO patients receiving norfloxacin than placebo at 1 month (7/8, 87.5 vs 0/7, p=0.004).	Ghoshal <i>et al.</i> <sup>114</sup>

SIBO, small intestinal bacterial overgrowth; GHBT, glucose hydrogen breath test; IBS, irritable bowel syndrome.

with nonconstipating IBS (n=1,260) diagnosed by Rome II criteria. IBS subjects receiving rifaximin at a dose of 550 mg three times daily for 14 days reported adequate relief in global IBS symptoms as compared to identical placebo (TARGET 1: 40.8% vs 31.2%, p=0.01 and TARGET 2: 40.6% vs 32.2%, p=0.03).<sup>115</sup> Moreover, rifaximin was more effective in relieving abdominal bloating than placebo (TARGET 1: 39.5% vs 28.7%, p=0.005 and TARGET 2: 41.0% vs 31.9%, p=0.02).<sup>115</sup> The improvement in symptoms of IBS (like abdominal pain, loose or watery stool) persisted for a duration of 10 weeks after the end of 2-week treatment.<sup>115</sup>

Recently, TARGET 3 study has been completed to evaluate the efficacy and safety of retreatment with rifaximin among 636 patients with IBS-D, who had responded to rifaximin previously but developed recurrent IBS symptoms over a duration of 18 weeks follow-up.<sup>116,117</sup> The end-point of TARGET 3 study was different from the TARGET 1 and TARGET 2 studies according to the guidelines proposed by Food and Drug Administration (FDA). TARGET 3 study included those IBS subjects who reported improvement in symptoms during at least 2 of the first 4 weeks in abdominal pain ( $\geq 30\%$  decrease from baseline in mean weekly pain score) and stool consistency ( $\geq 50\%$  decrease from baseline in the number of days per week with bowel movements complying with type 6 or 7 on the Bristol stool form scale).<sup>117,118</sup> Retreatments with rifaximin showed 33% response rate as compared to 25% in placebo group (p=0.02), consistent with FDA guidelines for clinical assessment of IBS drugs.<sup>118</sup>

In a study of open level antibiotic treatment, bacterial overgrowth was eradicated in 25 out of 47 patients and symptoms of IBS like diarrhea and abdominal pain were improved.<sup>40</sup> Moreover, 48% of the subjects were found negative for Rome I criteria.<sup>40</sup> In another study, of the 10 patients with SIBO treated with norfloxacin, amoxicillin-clavulanic acid and *Saccharomyces boulardii* over a period of 7 days,<sup>119</sup> norfloxacin and amoxicillin-clavulanic acid significantly improved the mean daily stool frequency, but not *S. boulardii*.<sup>119</sup> Recently, a proof of the concept study suggested that the lower response rate of 40% among patients treated with antibiotic in TARGET I and II studies might be related to the fact that patients were not selected based on the presence or absence of SIBO. In this study, 7 of 8 (87.5%) of 15 patients with SIBO treated with norfloxacin became Rome III negative at 1 month as compared to none of those treated with placebo. Interestingly, in this study, of 40 patients treated with norfloxacin, 15 (37.5%) responded showing that when not selected according to presence of SIBO, response rate was somewhat similar to the frequency of improvement as reported in TARGET I and II study.<sup>114</sup>

In a recent meta-analysis, efficiency of rifaximin (two studies) in eradicating SIBO was 64.1% as compared to 41% with other systemic antibiotics (metronidazole or tetracycline, p=0.003).<sup>120</sup> Another meta-analysis of eight studies showed that overall normalization rate of breath test with rifaximin was 49.5% (95%

CI, 44.0 to 55.1).<sup>121</sup> Antibiotics like metronidazole, neomycin and ciprofloxacin (four studies) showed higher response rate than placebo in normalizing breath tests with an odds ratio of 2.55 (95% CI, 1.29 to 5.04).<sup>121</sup> Thus, evidence from above studies suggests that antibiotics can be given in IBS patients with suspected SIBO.

## 2. Probiotics

Probiotics are live microorganisms, which, when administered in sufficient quantities may alleviate symptoms of IBS than placebo as shown by several clinical trials.<sup>2</sup> Probiotics may work by suppressing proinflammatory cytokines, modulating gut microbiota, sustaining the integrity of intestinal epithelium and altering the visceral hypersensitivity and brain function.<sup>28,122-125</sup> Randomized controlled trials of probiotics among patients with SIBO are scanty. An old randomized controlled cross-over study only on 10 patients with SIBO showed that though norfloxacin and amoxicillin-clavulanic acid were effective in improving mean daily stool frequency and breath hydrogen, *S. boulardii* administered for one week was ineffective.<sup>119</sup> Another study, however, showed that administration of high doses of *S. boulardii* for one month reduced abdominal pain, bloating, flatulence among pediatric patients with short bowel syndrome (SBS) and led to some change in bacterial flora in the stool samples suggesting that *S. boulardii* may impact the gut microbiota in patients with SBS.<sup>126</sup> Furthermore, probiotics may enhance the efficiency of antibiotics. One study showed that treatment with rifaximin along with probiotic (*Lactobacillus casei*) improved the symptoms of SIBO more effectively than antibiotic followed by prebiotic (short chain fructo-oligosaccharide).<sup>127</sup> Some studies recommended that treatment with rifaximin along with probiotics as a standard therapy for management of SIBO.<sup>127</sup> Use of multispecies probiotics had shown several benefits in relieving symptoms of IBS.<sup>128</sup> A randomized controlled trial of VSL#3 (twice daily for 8 weeks) in patients with IBS-D showed that abdominal bloating was significantly reduced as compared to placebo but not other parameters such as bowel dysfunction, colonic transit time, abdominal pain, flatulence or urgency.<sup>128,129</sup> More studies, however, are needed to evaluate efficacy of probiotics among patients with IBS in relation to presence of SIBO.

## 3. Prokinetics

Since IBS is associated with alteration and gut motility, and SIBO is associated with motility disorders, prokinetics are expected to be beneficial in patients with SIBO. In an earlier study, Pimentel *et al.*<sup>130</sup> showed that IBS patients with SIBO had lower frequency of migratory motor complex. Hence, it is expected that prokinetic drugs that improve small bowel motility might be useful in preventing SIBO following its successful treatment. The same group of authors showed that tegaserod, a serotonin receptor agonist, prevents the recurrence of IBS symptoms after antibiotic treatment compared to another prokinetic, erythro-

mycin (a motilin agonist).<sup>131</sup>

#### 4. Dietary manipulation of gut microbiota

Dietary manipulation may help patients with IBS in general and those with SIBO in particular.<sup>132,133</sup> In patients with SIBO, bacteria in the small bowel may ferment carbohydrates such as lactose, fructose and also the dietary fermentable oligo-, di-, monosaccharides and polyols (FODMAPs), which forms gas resulting in flatulence, abdominal bloating and pain.<sup>134,135</sup> Hence, restriction of these dietary components may improve these symptoms.<sup>136</sup> Moreover, some preliminary data suggest that manipulation of the diet may alter gut microbiota.<sup>136</sup> In a study, human fecal microbiota was transplanted into germ free mice that were fed low fat diet and plant polysaccharides.<sup>137</sup> Subsequently, feeding Western diet resulted in change in composition of gut microbiota leading to increased number of Firmicutes, *Clostridium* species, *Eubacterium*, *Enterococcus* and decreased number of *Bacteroides*.<sup>137</sup> Moreover, diet rich in complex carbohydrates favors growth of less pathogenic bacteria (*Mycobacterium avium* subspecies *paratuberculosis* and Enterobacteriaceae) than diet rich in fat or protein.<sup>138</sup> Vegetarian diets, rich in fiber, lead to higher production of short chain fatty acids, which inhibit potentially invasive bacteria like *E. coli* and other members of Enterobacteriaceae.<sup>138,139</sup> In a recent study, we found that vegetarianism was a risk factor for IBS on univariate and multivariate analysis.<sup>140</sup> More studies are needed to evaluate effect of dietary manipulation on gut microbiota including SIBO.

#### CONCLUSIONS

Recent realization that SIBO play an important role in pathogenesis of symptoms in a subset of patients with IBS led to a paradigm shift in understanding this disorder, hitherto thought to be predominantly psychogenic in nature. This is further substantiated by the initiative of Rome Foundation that introduced the concept of multidimensional clinical profile in diagnosis and management of functional GI disorders including IBS.<sup>141</sup> Though frequency of SIBO among patients with IBS varied between 4% and 78% patients, most studies reported the frequency to be higher among IBS than controls. Variation in the methods to diagnose SIBO is the most important reason for the wide variation in frequency of SIBO among patients with IBS in different studies. Quantitative jejunal aspirate culture, considered as the gold standard for the diagnosis of SIBO, is invasive and hence, hydrogen breath tests have been popularly used to diagnose SIBO.<sup>31,142</sup> However, whereas GHBT is highly specific, it is quite insensitive. On the other hand, the early-peak criteria in LHBT is highly nonspecific.<sup>36</sup> Hence, clinical phenotype of IBS may be used to consider treating patients empirically for possible SIBO. Diarrhea-predominant IBS (looser stool on Bristol scale), marked bloating and flatulence, older age, symptom development while on PPI therapy have been shown to be associated with SIBO

among patients with IBS<sup>143</sup>; unless better noninvasive methods for diagnosis of SIBO become available, patients with these clinical predictors may be treated for possible SIBO. Currently, rifaximin is the best treatment for SIBO among patients with IBS.

#### CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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