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ASSESSMENT OF GUT MICROBIOTA IN PATIENTS WITH IRRITABLE BOWEL SYNDROME: A COMPREHENSIVE ANALYSIS

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Abstract

This article presents a comprehensive assessment of gut microbiota composition and diversity in patients with irritable bowel syndrome (IBS). IBS is a common functional gastrointestinal disorder affecting 10-15% of the global population, with emerging evidence implicating gut microbiota dysbiosis in its pathophysiology. A case-control study of 120 IBS patients (40 IBS-D, 40 IBS-C, 40 IBS-M) and 40 healthy controls was conducted, employing 16S rRNA gene sequencing, metagenomic analysis, and metabolomic profiling. Comprehensive clinical assessment included Rome IV diagnostic criteria, symptom severity scoring, and quality of life evaluation. Results revealed significant microbiota alterations in IBS patients, including reduced alpha diversity (Shannon index 3.2±0.6 vs 4.1±0.5 in controls, $p<0.001$), decreased Firmicutes/Bacteroidetes ratio (1.8±0.4 vs 2.6±0.5, $p<0.001$), and reduced abundance of beneficial bacteria (Faecalibacterium prausnitzii, Bifidobacterium spp). IBS-D showed increased Enterobacteriaceae (12.4±3.2% vs 3.8±1.2%, $p<0.001$), while IBS-C demonstrated reduced Prevotella abundance. Metabolomic analysis identified altered short-chain fatty acid profiles and increased fecal calprotectin levels. The discussion emphasizes the gut-brain axis, immune modulation, and therapeutic implications including probiotics, prebiotics, fecal microbiota transplantation, and dietary interventions.

Keywords

irritable bowel syndrome, gut microbiota, dysbiosis, 16S rRNA sequencing, metagenomics, short-chain fatty acids, gut-brain axis, probiotics.

INTRODUCTION

Irritable bowel syndrome (IBS) represents one of the most prevalent functional gastrointestinal disorders, affecting approximately 10-15% of the global population with significant impact on quality of life and healthcare costs [1]. Characterized by recurrent abdominal pain associated with altered bowel habits, IBS is classified into subtypes based on predominant stool pattern: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), IBS with mixed bowel habits (IBS-M), and unclassified IBS (IBS-U) according to Rome IV criteria [2]. Despite its high prevalence, the pathophysiology of IBS remains incompletely understood, with contributions from visceral hypersensitivity, altered gut motility, psychosocial factors, and immune activation.

Recent advances in microbiome research have illuminated the critical role of gut microbiota in IBS pathogenesis. The human gastrointestinal tract harbors approximately 100 trillion microorganisms representing over 1,000 species, collectively termed the gut microbiota [3]. This complex microbial ecosystem performs essential functions including nutrient metabolism, vitamin synthesis, immune system modulation, pathogen resistance, and production of neuroactive metabolites. Dysbiosis, defined as alterations in microbiota composition and function, has been consistently observed in IBS patients across multiple studies [4].

The microbiota-gut-brain axis, a bidirectional communication network linking the gut microbiome with the central nervous system, provides a mechanistic framework for understanding IBS symptomatology [5]. Gut bacteria produce neurotransmitters (serotonin, gamma-aminobutyric acid), short-chain fatty acids (SCFAs), and other metabolites that influence intestinal function, immune responses, and neural signaling. Dysbiosis-induced alterations in these pathways may contribute to visceral hypersensitivity, dysmotility, and the psychological comorbidities frequently observed in IBS patients [6].

Previous studies have identified several microbiota alterations in IBS, including reduced diversity, decreased abundance of beneficial bacteria (particularly Lactobacillus and Bifidobacterium species), and increased potentially pathogenic organisms. However, results have been inconsistent across studies due to methodological heterogeneity, small sample sizes, and geographic variations [7]. Furthermore, subtype-specific microbiota patterns require clarification to enable personalized therapeutic approaches.

Therapeutic modulation of gut microbiota through probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT) has shown promise in IBS management, though efficacy varies considerably . Understanding the specific microbiota alterations in IBS subtypes is essential for developing targeted interventions and predicting treatment response.

The aim of this research is to comprehensively assess gut microbiota composition, diversity, and functional capacity in IBS patients compared to healthy controls, and to identify subtype-specific microbiota signatures. Specific objectives include: characterizing microbiota composition using 16S rRNA sequencing and metagenomic analysis, evaluating alpha and beta diversity metrics, identifying differentially abundant bacterial taxa in IBS subtypes, analyzing metabolomic profiles including short-chain fatty acids, and correlating microbiota features with clinical parameters and symptom severity.

MATERIALS AND METHODS

Study design and participants. This case-control study was conducted at the Gastroenterology Department from January 2022 to December 2024. A total of 120 IBS patients diagnosed according to Rome IV criteria were enrolled: 40 IBS-D, 40 IBS-C, and 40 IBS-M patients. Rome IV criteria require recurrent abdominal pain (≥1 day per week in the last 3 months) associated with ≥2 of: defecation, change in stool frequency, or change in stool form, with symptom onset ≥6 months prior . Forty age- and gender-matched healthy controls without gastrointestinal symptoms were recruited. Exclusion criteria included: inflammatory bowel disease, celiac disease, colorectal cancer, recent antibiotic use (<3 months), probiotic supplementation (<1 month), pregnancy, lactation, and severe systemic diseases. All participants provided written informed consent. The study protocol was approved by the institutional ethics committee (Protocol #2021-456).

Clinical assessment. Comprehensive clinical evaluation included detailed medical history, physical examination, and completion of validated questionnaires. IBS symptom severity was assessed using the IBS Severity Scoring System (IBS-SSS), with scores ranging from 0-500 (mild <175, moderate 175-300, severe >300) . Quality of life was evaluated using the IBS Quality of Life (IBS-QOL) questionnaire. Anxiety and depression were screened using the Hospital Anxiety and Depression Scale (HADS). Bowel habit patterns were documented using the Bristol Stool Form Scale. Blood tests including complete blood count, C-reactive protein, and celiac serology were performed to exclude organic pathology.

Fecal sample collection and processing. Fecal samples were collected using sterile collection kits with DNA stabilization buffer and transported on ice within 4 hours. Samples were aliquoted and stored at -80°C until analysis. DNA extraction was performed using the QIAamp DNA Stool Mini Kit (Qiagen) following manufacturer protocols with bead-beating step for enhanced bacterial cell lysis. DNA concentration and purity were assessed by spectrophotometry (A260/A280 ratio 1.8-2.0).

16S rRNA gene sequencing. The V3-V4 hypervariable regions of bacterial 16S rRNA genes were amplified using universal primers (341F/805R) and sequenced on Illumina MiSeq platform generating 2×300 bp paired-end reads. Bioinformatic analysis employed QIIME2 pipeline . Raw sequences were quality-filtered, denoised using DADA2, and clustered into amplicon sequence variants (ASVs). Taxonomic classification was performed using SILVA database (v138). Alpha diversity metrics (Shannon index, Simpson index, Chao1 richness) and beta diversity (weighted and unweighted UniFrac distances) were calculated.

Shotgun metagenomic sequencing. Shotgun metagenomic sequencing was performed on a subset of 40 samples (10 from each group) using Illumina NovaSeq platform. Metagenomic reads were quality-controlled, host DNA filtered, and taxonomically profiled using MetaPhlAn3. Functional profiling was conducted using HUMAnN3 to identify metabolic pathways and gene families. KEGG pathway analysis assessed functional capacity differences between groups.

Metabolomic analysis. Fecal short-chain fatty acid (SCFA) concentrations (acetate, propionate, butyrate) were quantified by gas chromatography-mass spectrometry (GC-MS). Samples were homogenized, acidified, and extracted with diethyl ether. Internal standards were added for quantification. Fecal calprotectin, a marker of intestinal inflammation, was measured by ELISA. Bile acid profiles were analyzed by liquid chromatography-mass spectrometry (LC-MS).

Statistical analysis. Data were analyzed using R software version 4.3.0. Continuous variables were presented as mean ± standard deviation or median (interquartile range). Group comparisons employed Student t-test, Mann-Whitney U test, or Kruskal-Wallis test as appropriate. Alpha diversity indices were compared using Wilcoxon rank-sum test. Beta diversity differences were assessed by PERMANOVA. Differentially abundant taxa were identified using LEfSe (Linear discriminant analysis Effect Size) with LDA score >2.0. Correlation analyses used Spearman correlation coefficients. Multiple testing correction applied the Benjamini-Hochberg false discovery rate method. P-values <0.05 were considered statistically significant.

RESULTS

Clinical characteristics. The study cohort comprised 160 participants: 120 IBS patients (72 females, 48 males) with mean age 38.6±12.4 years and 40 healthy controls (24 females, 16 males) with mean age 37.8±11.2 years. Disease duration averaged 4.8±3.2 years. IBS-SSS scores were: IBS-D 286±64, IBS-C 268±72, IBS-M 294±58, indicating moderate-to-severe symptoms across subtypes. Quality of life was significantly impaired in IBS patients (IBS-QOL score 58±18) compared to controls (92±8, $p<0.001$). Anxiety (HADS-A ≥8) was present in 58% of IBS patients versus 12% of controls ($p<0.001$). Depression (HADS-D ≥8) affected 42% of IBS patients and 8% of controls ($p<0.001$) .

Alpha diversity analysis. IBS patients demonstrated significantly reduced alpha diversity compared to controls. Shannon diversity index was 3.2±0.6 in IBS patients versus 4.1±0.5 in controls ($p<0.001$). Simpson index showed similar reduction (0.82±0.08 vs 0.92±0.04, $p<0.001$). Species richness (Chao1) was lower in IBS (186±42 vs 268±38, $p<0.001$). Among IBS subtypes, IBS-D showed the lowest diversity (Shannon 2.9±0.5), followed by IBS-M (3.2±0.6) and IBS-C

(3.4±0.6), though inter-subtype differences were not statistically significant (p=0.08). These findings indicate overall microbiota depletion in IBS patients .

Beta diversity and community structure. Principal coordinate analysis (PCoA) of weighted UniFrac distances revealed significant separation between IBS patients and controls (PERMANOVA R^2=0.18, p<0.001), indicating distinct microbiota community structures. IBS subtypes clustered separately from controls but showed partial overlap with each other, suggesting both shared and subtype-specific microbiota features. The Firmicutes/Bacteroidetes ratio, a major structural indicator, was significantly reduced in IBS patients (1.8±0.4) compared to controls (2.6±0.5, p<0.001), reflecting altered phylum-level composition.

Taxonomic composition differences. At the phylum level, IBS patients showed increased Proteobacteria (8.6±3.4% vs 3.2±1.8% in controls, p<0.001) and decreased Actinobacteria (2.4±1.2% vs 5.8±2.4%, p<0.001). At the family level, Enterobacteriaceae was elevated in IBS-D (12.4±3.2% vs 3.8±1.2% in controls, p<0.001), while Ruminococcaceae was reduced across all IBS subtypes (8.2±3.6% vs 16.4±4.2%, p<0.001). At the genus level, beneficial bacteria showed marked depletion: Faecalibacterium (4.2±2.4% vs 12.8±3.6%, p<0.001), Bifidobacterium (1.8±1.2% vs 6.4±2.2%, p<0.001), and Roseburia (2.6±1.4% vs 7.2±2.4%, p<0.001). Conversely, potentially pathogenic genera were enriched in IBS: Escherichia-Shigella (6.8±2.4% vs 1.2±0.8%, p<0.001) and Klebsiella (3.4±1.8% vs 0.6±0.4%, p<0.001) .

Subtype-specific microbiota signatures. LEfSe analysis identified distinctive microbial biomarkers for each IBS subtype. IBS-D was characterized by enrichment of Proteobacteria, particularly Enterobacteriaceae family and Escherichia genus. IBS-C showed reduced abundance of Prevotella (2.4±1.6% vs 8.6±3.2% in controls, p<0.001) and Akkermansia muciniphila (0.8±0.6% vs 3.2±1.4%, p=0.002). IBS-M demonstrated intermediate patterns with elements of both IBS-D and IBS-C profiles. Methanobrevibacter smithii, a methanogenic archaeon associated with constipation, was significantly elevated in IBS-C (4.2±2.1% vs 1.4±0.8%, p<0.001).

Functional metagenomic analysis. Metagenomic profiling revealed altered functional capacity in IBS patients. Genes involved in carbohydrate metabolism, particularly those encoding enzymes for complex polysaccharide degradation, were reduced in IBS (p<0.001). Bile acid metabolism pathways showed significant alterations, with decreased bile salt hydrolase activity in IBS patients. Pathways related to lipopolysaccharide biosynthesis were enriched in IBS-D, consistent with increased Gram-negative bacteria. Interestingly, genes encoding tryptophan metabolism enzymes, relevant for serotonin production, were altered in IBS patients compared to controls .

Short-chain fatty acid profiles. Fecal SCFA concentrations were significantly reduced in IBS patients. Total SCFA levels were 62.4±18.6 mmol/kg in IBS versus 94.8±22.4 mmol/kg in controls (p<0.001). Butyrate, a key metabolite for colonocyte health, showed the most pronounced reduction (12.4±4.8 mmol/kg vs 28.6±8.2 mmol/kg, p<0.001). Propionate and acetate were also decreased. Among IBS subtypes, IBS-C exhibited the lowest butyrate levels (9.8±3.6 mmol/kg, p<0.001 vs controls), correlating with reduced butyrate-producing bacteria (Faecalibacterium, Roseburia). The butyrate reduction correlated with symptom severity (r=-0.52, p<0.001) and impaired quality of life (r=0.48, p<0.001) .

Inflammatory markers. Fecal calprotectin, while remaining within normal range (<50 μg/g), was significantly elevated in IBS patients (38.6±16.4 μg/g) compared to controls (18.2±8.4 μg/g, p<0.001), suggesting low-grade intestinal inflammation. IBS-D patients had higher calprotectin levels (46.8±18.2 μg/g) than IBS-C (32.4±14.6 μg/g, p=0.008). Calprotectin correlated positively

with Enterobacteriaceae abundance (r=0.42, p<0.001) and negatively with Faecalibacterium (r=-0.38, p<0.001).

Correlations with clinical parameters. Microbiota diversity indices correlated inversely with IBS symptom severity (Shannon index vs IBS-SSS: r=-0.46, p<0.001). Faecalibacterium abundance correlated negatively with abdominal pain severity (r=-0.42, p<0.001). Bifidobacterium levels correlated positively with quality of life scores (r=0.38, p=0.002). Anxiety scores correlated with Enterobacteriaceae abundance (r=0.34, p=0.006). These correlations suggest that microbiota composition influences clinical manifestations and psychological comorbidities in IBS.

DISCUSSION

This comprehensive microbiota assessment demonstrates significant dysbiosis in IBS patients, characterized by reduced diversity, altered taxonomic composition, diminished butyrate-producing bacteria, and subtype-specific signatures. These findings advance our understanding of IBS pathophysiology and have important therapeutic implications.

Microbiota diversity and community structure. The observed reduction in alpha diversity (Shannon index 3.2 vs 4.1 in controls) aligns with previous reports and suggests impaired ecosystem stability in IBS . Reduced diversity may compromise functional redundancy, making the microbiota more vulnerable to perturbations and less capable of maintaining homeostatic functions. The decreased Firmicutes/Bacteroidetes ratio (1.8 vs 2.6) represents a major phylum-level shift with implications for nutrient metabolism and immune function. This pattern contrasts with obesity-associated dysbiosis, which typically shows increased F/B ratio, highlighting IBS-specific microbiota signatures.

The depletion of beneficial bacteria, particularly Faecalibacterium prausnitzii and Bifidobacterium species, is particularly significant. F. prausnitzii is a major butyrate producer with anti-inflammatory properties, and its reduction has been linked to compromised intestinal barrier function and increased permeability. Bifidobacterium species confer multiple benefits including pathogen exclusion, immune modulation, and production of beneficial metabolites. Their depletion may contribute to IBS symptomatology through multiple pathways.

Subtype-specific microbiota patterns and mechanisms. The enrichment of Enterobacteriaceae in IBS-D (12.4% vs 3.8% in controls) provides mechanistic insights into diarrhea pathogenesis. Enterobacteriaceae are Gram-negative bacteria producing lipopolysaccharide (LPS), a potent immune activator that can trigger inflammation and increase intestinal permeability. LPS may stimulate visceral afferent neurons, contributing to visceral hypersensitivity and pain. Additionally, certain Enterobacteriaceae produce hydrogen sulfide, which accelerates colonic transit and may contribute to diarrhea .

The reduction of Prevotella and Akkermansia muciniphila in IBS-C offers potential mechanistic explanations for constipation. A. muciniphila degrades mucin, stimulating mucus production and maintaining the mucus layer integrity. Its depletion may result in thinner mucus layer and slower transit. The elevation of Methanobrevibacter smithii in IBS-C is particularly interesting, as methane production has been associated with slower transit through effects on smooth muscle contractility . Methane may also alter water absorption, contributing to constipation.

Short-chain fatty acids and intestinal homeostasis. The marked reduction in fecal butyrate (12.4 vs 28.6 mmol/kg) has multiple implications. Butyrate is the preferred energy source for colonocytes and exerts anti-inflammatory effects through inhibition of histone deacetylases and activation of GPR43/GPR109A receptors. Butyrate deficiency may impair epithelial barrier

function, increase permeability ("leaky gut"), and promote low-grade inflammation . The correlation between butyrate levels and symptom severity (r=-0.52) suggests therapeutic potential for butyrate supplementation or strategies to enhance butyrate-producing bacteria.

Propionate and acetate reductions also contribute to dysbiosis consequences. These SCFAs regulate intestinal motility, modulate immune responses, and serve as substrates for lipid and glucose metabolism. The overall SCFA deficit in IBS represents loss of multiple beneficial functions essential for intestinal and systemic health.

Gut-brain axis and neuropsychological connections. The correlations between microbiota composition and anxiety/depression scores support the role of the microbiota-gut-brain axis in IBS. Gut bacteria influence brain function through multiple pathways: production of neurotransmitters (90% of body serotonin is produced in the gut), modulation of vagal nerve signaling, effects of bacterial metabolites on neuroendocrine pathways, and immune-mediated mechanisms affecting neuroinflammation . The altered tryptophan metabolism pathways identified in metagenomic analysis are particularly relevant, as tryptophan is the precursor for serotonin synthesis.

The high prevalence of anxiety (58%) and depression (42%) in our IBS cohort, combined with microbiota-psychology correlations, suggests that microbiota-targeted therapies might address both gastrointestinal and psychological symptoms. Psychobiotics, defined as live organisms that when ingested in adequate amounts produce mental health benefits, represent a promising therapeutic avenue.

Therapeutic implications. Our findings support several therapeutic strategies. Probiotic supplementation targeting depleted beneficial species (Bifidobacterium, Lactobacillus, Faecalibacterium) shows promise, though strain-specific effects require consideration. Meta-analyses demonstrate modest but significant benefits of probiotics in IBS, with certain multi-strain formulations achieving superior efficacy . Prebiotic fibers promoting growth of butyrate-producing bacteria may address SCFA deficiency. The low-FODMAP diet, while effective for symptom management, may further reduce beneficial bacteria and requires careful implementation with gradual reintroduction.

Fecal microbiota transplantation (FMT) has shown variable results in IBS trials, with some studies reporting significant improvements while others found no benefit. Our identification of specific dysbiosis patterns may enable better patient selection for FMT, targeting those with most severe microbiota depletion. Rifaximin, a minimally absorbed antibiotic, has demonstrated efficacy in IBS-D, potentially through reduction of pathogenic bacteria like Enterobacteriaceae, though careful monitoring for dysbiosis exacerbation is warranted .

Limitations and future directions. Study limitations include cross-sectional design precluding causal inferences, single geographic location potentially limiting generalizability, and reliance on fecal samples which may not fully reflect small intestinal microbiota. Future research should employ longitudinal designs to assess microbiota stability and temporal relationships with symptoms, expand to multi-center studies across diverse populations, investigate small intestinal microbiota through aspiration or capsule technologies, and conduct randomized controlled trials of microbiota-targeted interventions guided by baseline microbiota profiling.

CONCLUSION

This comprehensive microbiota assessment in IBS patients reveals significant dysbiosis with important pathophysiological and therapeutic implications. Principal conclusions include:

1) IBS patients exhibit marked gut microbiota dysbiosis characterized by reduced alpha diversity (Shannon index 3.2 vs 4.1), decreased Firmicutes/Bacteroidetes ratio (1.8 vs 2.6), and altered community structure compared to healthy controls. This dysbiosis represents a consistent feature across IBS subtypes.

2) Beneficial bacteria, particularly Faecalibacterium prausnitzii and Bifidobacterium species, are significantly depleted in IBS, while potentially pathogenic bacteria including Enterobacteriaceae are enriched. This imbalance contributes to reduced anti-inflammatory capacity and compromised intestinal barrier function.

3) Subtype-specific microbiota signatures exist, with IBS-D showing elevated Enterobacteriaceae and IBS-C demonstrating reduced Prevotella and increased Methanobrevibacter. These patterns provide mechanistic insights into symptom generation and suggest opportunities for personalized interventions.

4) Short-chain fatty acid deficiency, particularly butyrate reduction (12.4 vs 28.6 mmol/kg), correlates with symptom severity and quality of life impairment. Restoring SCFA production through dietary or microbial interventions represents a rational therapeutic target.

5) Microbiota composition correlates with clinical parameters including symptom severity, quality of life, and psychological comorbidities, supporting the microbiota-gut-brain axis involvement in IBS pathophysiology. Microbiota-targeted therapies addressing both gastrointestinal and neuropsychological symptoms warrant investigation.

Understanding gut microbiota alterations in IBS provides a foundation for developing targeted therapeutic strategies including probiotics, prebiotics, dietary modifications, and potentially fecal microbiota transplantation. Future research should focus on longitudinal microbiota dynamics, mechanistic studies elucidating causality, and randomized controlled trials evaluating microbiota-directed interventions. Personalized medicine approaches based on individual microbiota profiles may optimize treatment selection and improve outcomes in this challenging disorder.

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