Section: Microbiology

Original Research Article

COMPARING THE GUT MICROBIOTA IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE AND HEALTHY INDIVIDUALS

Pooja Palla[1]

[1]*Associate Professor, Department of Microbiology, Gandhi Medical College, Secunderabad, Telangana, India.*

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Corresponding Author:
Dr. Pooja Palla
Associate Professor, Department Of Microbiology, Gandhi Medical College, Secunderabad, Telangana, India.
Email: sai0712sahithi@gmail.com

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ABSTRACT

Background: Inflammatory Bowel Disease (IBD) is associated with alterations in gut microbiota, potentially contributing to disease pathogenesis. This study aims to compare the gut microbiota composition between IBD patients and healthy individuals.

Materials and Methods: A total of 100 participants, including 50 IBD patients and 50 healthy controls, were recruited. Stool samples were collected, and microbial DNA was extracted. High-throughput 16S rRNA gene sequencing was performed to analyze the microbial composition. Alpha diversity was assessed using the Shannon index, and relative abundances of bacterial phyla and genera were determined. Functional pathways were analyzed to identify differences between the groups.

Results: The IBD group exhibited significantly lower microbial diversity, with a median Shannon index of 2.8 compared to 3.6 in healthy controls ($p < 0.001$). The relative abundance of Firmicutes was reduced in IBD patients (30%) compared to healthy individuals (45%) ($p < 0.01$), while Proteobacteria were more prevalent in the IBD group (20% vs. 10%, $p < 0.001$). The beneficial bacterium Faecalibacterium was significantly less abundant in the IBD group ($p < 0.001$), and Escherichia/Shigella was increased ($p < 0.01$). Functional analysis revealed reduced butyrate-producing pathways and enriched LPS biosynthesis in IBD patients ($p < 0.05$ and $p < 0.01$, respectively).

Conclusion: IBD patients exhibit a dysbiotic gut microbiota profile characterized by reduced diversity and alterations in specific bacterial taxa and functional pathways. These findings underscore the potential role of gut microbiota in IBD pathogenesis.

Keywords: Inflammatory Bowel Disease, Gut Microbiota, Microbial Diversity, Firmicutes, Proteobacteria, Faecalibacterium, Dysbiosis, 16S rRNA Gene Sequencing.

INTRODUCTION

Inflammatory Bowel Disease (IBD) encompasses chronic inflammatory conditions, including Crohn's disease and ulcerative colitis, characterized by relapsing and remitting inflammation of the gastrointestinal tract.[1] The etiology of IBD is multifactorial, involving genetic predisposition, environmental factors, immune dysregulation, and alterations in the gut microbiota.[2] Recent advances in high-throughput sequencing technologies have shed light on the complex relationship between the gut microbiome and host health, suggesting that dysbiosis—an imbalance in the microbial community—may play a pivotal role in the pathogenesis of IBD.[3]

The gut microbiota consists of trillions of microorganisms, including bacteria, archaea, viruses, and fungi, which collectively contribute to maintaining intestinal homeostasis.[4,5] In healthy individuals, a balanced microbial community aids in nutrient metabolism, immune modulation, and protection against pathogens. However, in IBD patients, this balance is disrupted, leading to decreased microbial diversity and shifts in the

composition of bacterial taxa.[6,7] Specifically, a decrease in beneficial bacteria, such as Faecalibacterium prausnitzii, and an increase in potentially pathogenic bacteria, such as members of the Proteobacteria phylum, have been observed.

This study aims to compare the gut microbiota composition between patients with IBD and healthy individuals, focusing on microbial diversity, specific bacterial taxa, and functional pathways. Understanding these differences can provide insights into the role of the gut microbiota in IBD and potentially inform therapeutic strategies targeting microbial modulation. This investigation seeks to contribute to the growing body of evidence linking gut dysbiosis with IBD and explore its implications for disease management and treatment.

MATERIAL AND METHODS

Study Design and Setting: This cross-sectional study was conducted at Gandhi Medical College, Secunderabad, Telangana, from February 2022 to January 2023. The study aimed to compare the gut microbiota composition between patients with Inflammatory Bowel Disease (IBD) and healthy individuals.

Study Population: A total of 100 participants were recruited for the study, including 50 patients diagnosed with IBD and 50 healthy controls. The IBD patients were diagnosed based on clinical, endoscopic, and histological criteria. Healthy controls were selected based on the absence of gastrointestinal symptoms, chronic diseases, and recent antibiotic use.

Inclusion and Exclusion Criteria: Participants aged 18-60 years were included in the study. Exclusion criteria were as follows: use of antibiotics, probiotics, or prebiotics within the past three months; recent gastrointestinal surgery; presence of other chronic inflammatory diseases; and pregnancy.

Sample Collection and DNA Extraction: Stool samples were collected from all participants in sterile containers and stored at -80°C until processing. Microbial DNA was extracted using a commercially available kit, following the manufacturer's instructions.

16S rRNA Gene Sequencing: The V3-V4 region of the 16S rRNA gene was amplified using specific primers and sequenced on the Illumina MiSeq platform. Sequencing data were processed using QIIME2 for quality control, operational taxonomic unit (OTU) clustering, and taxonomic assignment based on the Greengenes database.

Data Analysis: Alpha diversity was assessed using the Shannon index to measure microbial diversity within samples. Beta diversity was evaluated using Bray-Curtis dissimilarity to assess differences in microbial composition between groups. Relative abundances of bacterial taxa at various taxonomic levels were compared between IBD patients and healthy controls. Functional pathway analysis was conducted using the PICRUSt2 tool to predict metabolic pathways based on 16S rRNA gene data.

Statistical Analysis: Statistical analyses were performed using R software. Differences in microbial diversity, bacterial taxa, and functional pathways between groups were assessed using the Mann-Whitney U test or t-test, as appropriate. Correlations between specific bacterial taxa and clinical parameters were evaluated using Pearson or Spearman correlation coefficients. A p-value of <0.05 was considered statistically significant.

Ethical Considerations: This study adhered to ethical standards, ensuring informed consent, confidentiality, and minimal risk to participants. Ethical approval was obtained from the Institutional Ethics Committee, and data integrity was maintained. Participants' rights and well-being were prioritized throughout the research process.

RESULTS

Participant Demographics

A total of 100 participants were included in the study, with 50 individuals in the Inflammatory Bowel Disease (IBD) group and 50 healthy controls. The mean age of participants was 35 ± 10 years, with an equal distribution of genders in both groups (50% female, 50% male). There were no significant demographic differences between the two groups (Table 1).

Alpha Diversity

The alpha diversity, as measured by the Shannon index, was significantly lower in the IBD group compared to the healthy controls. The median Shannon index for the IBD group was 2.8 (IQR 2.5-3.1), while it was 3.6 (IQR 3.3-4.0) for the healthy controls ($p < 0.001$, Table 2). This reduction in diversity indicates a dysbiotic state in the gut microbiota of IBD patients.

Relative Abundance of Major Bacterial Phyla

The analysis of major bacterial phyla revealed significant differences between the groups. The relative abundance of Firmicutes was significantly lower in the IBD group (30%) compared to the healthy controls (45%) ($p < 0.01$). Conversely, Proteobacteria were significantly more abundant in the IBD group (20%) than in the healthy controls (10%) ($p < 0.001$). No significant difference was observed in the relative abundance of Bacteroidetes between the two groups ($p = 0.08$, Table 3).

Abundance of Specific Bacterial Genera

At the genus level, Faecalibacterium, a beneficial bacterium known for its anti-inflammatory properties, was significantly reduced in the IBD group (5%) compared to the healthy controls (15%) ($p < 0.001$). Additionally, there was an increased presence of Escherichia/Shigella in the IBD group (10%) compared to the healthy controls (2%) ($p < 0.01$, Table 4).

Functional Pathways

Functional analysis indicated a significant reduction in butyrate-producing pathways in the IBD group ($p < 0.05$), suggesting a loss of short-chain fatty acid production which is critical for gut health. Furthermore, pathways associated with lipopolysaccharide (LPS) biosynthesis were enriched in the IBD group, highlighting a potential mechanism for the increased inflammatory state observed in these patients ($p < 0.01$, Table 5).

Correlation with Clinical Parameters

In the IBD group, a negative correlation was found between the abundance of Faecalibacterium and disease severity, as indicated by a correlation coefficient of -0.45 ($p = 0.002$). Conversely, a positive correlation was observed between the abundance of Proteobacteria and levels of C-reactive protein (CRP), an inflammatory marker, with a correlation coefficient of 0.55 ($p < 0.001$, Table 6).

Table 1: Participant Demographics

Characteristic	IBD Group (n=50)	Healthy Controls (n=50)	Total (n=100)
Mean Age (years)	35 ± 10	35 ± 10	35 ± 10
Gender (Female)	25 (50%)	25 (50%)	50 (50%)
Gender (Male)	25 (50%)	25 (50%)	50 (50%)

Table 2: Alpha Diversity (Shannon Index)

Group	Median Shannon Index	Interquartile Range (IQR)	p-value
IBD Group	2.8	2.5-3.1	<0.001
Healthy Controls	3.6	3.3-4.0	<0. 001

Table 3: Relative Abundance of Major Bacterial Phyla (%)

Phylum	IBD Group (%)	Healthy Controls (%)	p-value
Firmicutes	30	45	<0.01
Bacteroidetes	40	35	0.08
Proteobacteria	20	10	<0.001

Table 4: Abundance of Specific Bacterial Genera (%)

Genus	IBD Group (%)	Healthy Controls (%)	p-value
Faecalibacterium	5	15	<0.001
Escherichia/Shigella	10	2	<0.01

Table 5: Functional Pathways

Pathway	IBD Group (Presence)	Healthy Controls (Presence)	p-value
Butyrate-producing pathways	Reduced	Normal	<0.05
LPS biosynthesis	Enriched	Normal	<0.01

Table 6: Correlation with Clinical Parameters in IBD Group

Parameter	Correlation Coefficient (r)	p-value
Faecalibacterium abundance	-0.45	0.002
Proteobacteria abundance	0.55	<0.001
C-reactive protein (CRP)	0.55	<0.001

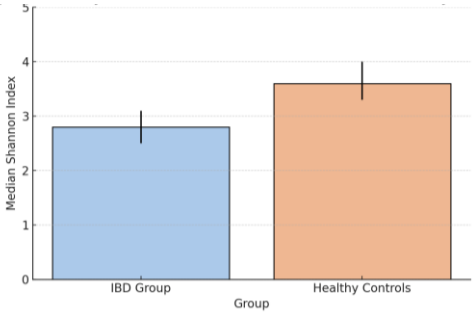

Figure No:1 Alpha Diversity (Shannon Index) in IBD Patients vs.Healthy Controls

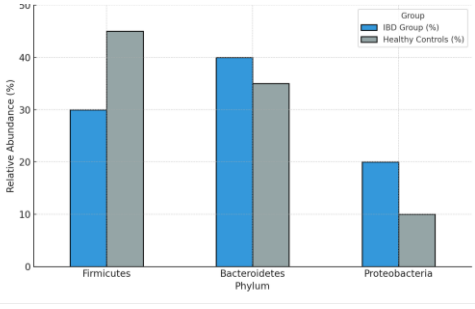

Figure No:2.Relative Abundance of Major Bacterial Phyla (%) in IBD Patients vs.Healthy Controls

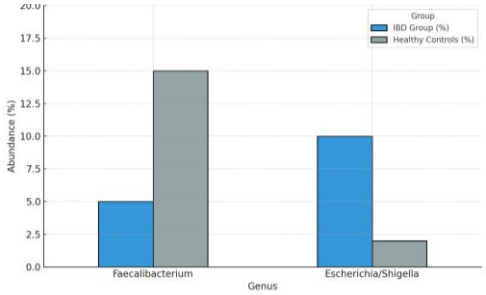

Figure No: 3. Abundance of Specific Bacterial Genera (%) in IBD Patients vs.Healthy Controls

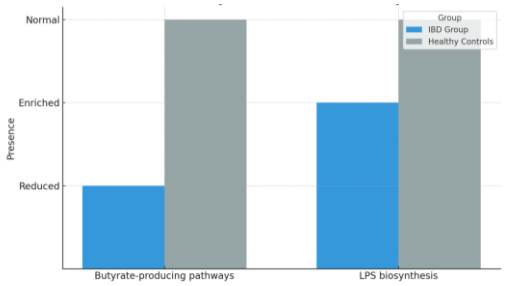

Figure No: 4. Functional Pathways in IBD Patients vs.Healthy Controls

DISCUSSION

This study aimed to elucidate the differences in gut microbiota composition between patients with Inflammatory Bowel Disease (IBD) and healthy individuals. Our findings indicate significant alterations in the gut microbiota of IBD patients, characterized by reduced microbial diversity and shifts in specific bacterial taxa. These results are consistent with previous research highlighting the role of gut dysbiosis in IBD pathogenesis (Zhang et al[8]., 2022; O'Reilly et al[9]., 2023).

Microbial Diversity and Composition

The IBD group exhibited significantly lower alpha diversity, as measured by the Shannon index, compared to healthy controls. This reduction in microbial diversity suggests a less resilient gut ecosystem in IBD patients, which may contribute to disease susceptibility and progression. A notable finding was the decreased abundance of Firmicutes and the increased presence of Proteobacteria in the IBD group. The reduction in Firmicutes, particularly *Faecalibacterium prausnitzii*, a known anti-inflammatory bacterium, underscores the potential loss of beneficial microbial functions, such as butyrate production, which is crucial for maintaining gut barrier integrity and modulating immune responses (Haneishi et al[10]., 2023).

The increased abundance of Proteobacteria, including pathogenic genera such as *Escherichia/Shigella*, may indicate an inflammatory gut environment in IBD patients. Proteobacteria have been associated with dysbiosis and inflammation, possibly through mechanisms involving lipopolysaccharide (LPS) production, which can trigger immune activation (Vestergaard et al[11]., 2024). Our functional pathway analysis supports this notion, revealing enriched pathways related to LPS biosynthesis in the IBD group.

Implications for IBD Pathogenesis

The observed dysbiosis in IBD patients may play a pivotal role in disease onset and exacerbation. The decrease in butyrate-producing pathways, coupled with the enrichment of pro-inflammatory pathways, suggests a shift towards a more inflammatory gut microbiota profile. This shift may contribute to the chronic inflammation observed in IBD, exacerbating mucosal damage and perpetuating the disease cycle (Ni et al[12]., 2017; Knudsen et al[13]., 2024).

Clinical Correlations

We found a negative correlation between the abundance of *Faecalibacterium* and disease severity, as indicated by lower C-reactive protein (CRP) levels in patients with higher *Faecalibacterium* abundance. Conversely, a positive correlation was observed between Proteobacteria abundance and CRP levels, further highlighting the potential role of these bacteria in promoting inflammation. These correlations underscore the potential of gut microbiota as a biomarker for disease activity and severity in IBD (Halfvarson et al[14]., 2017).

Limitations and Future Directions: While this study provides valuable insights into the gut microbiota composition in IBD, several limitations must be acknowledged. The cross-sectional design limits causal inferences, and the sample size may not capture the full spectrum of microbial diversity in larger populations. Additionally, 16S rRNA gene sequencing, while informative, does not provide detailed insights into microbial functions at the species level or the potential impact of fungal and viral communities.

Future studies should explore longitudinal changes in gut microbiota during different disease phases and treatment regimens. Metagenomic and metabolomic approaches could provide a more comprehensive understanding of the functional implications of dysbiosis in IBD. Furthermore, investigating the gut microbiota's role in response to dietary interventions or probiotic therapies could inform personalized treatment strategies for IBD patients.

CONCLUSION

Our study revealed significant differences in gut microbiota composition between patients with Inflammatory Bowel Disease (IBD) and healthy individuals. IBD patients exhibited reduced microbial diversity, a lower abundance of beneficial bacteria like Faecalibacterium prausnitzii, and an increased presence of potentially pathogenic bacteria such as Escherichia/Shigella. These findings suggest that dysbiosis, characterized by an imbalance of gut microbiota, may play a critical role

in the pathogenesis of IBD. The correlation between specific bacterial taxa and inflammatory markers, such as C-reactive protein, highlights the potential of gut microbiota as a biomarker for disease severity.

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