Section: Miscellaneous

Original Research Article

MICROBIOME DYSBIOSIS AND ITS ASSOCIATION WITH INFLAMMATORY BOWEL DISEASE: A CROSS-SECTIONAL INVESTIGATION OF GUT MICROBIOTA IN PATIENTS WITH CHRONIC INFLAMMATION

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Received *: 20/12/2024*
Received in revised form *: 02/02/2025*
Accepted *: 17/02/2025*

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DOI: 10.70034/ijmedph.2025.1.133

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (1); 710-717

ABSTRACT

Background: Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract influenced by genetic, environmental, immune, and microbial factors. Gut microbiome dysbiosis, characterized by an imbalance between beneficial and pathogenic bacteria, plays a crucial role in disease pathogenesis. This study aimed to investigate microbial alterations, inflammatory biomarkers, and histopathological changes in IBD patients compared to healthy controls, with a focus on differentiating CD from UC.

Materials and Methods: This cross-sectional study was conducted over two years at a tertiary healthcare institution in India, enrolling 124 IBD patients (62 CD and 62 UC) and 124 healthy controls, matched for age, sex, and dietary habits. Fecal samples were analyzed using 16S rRNA sequencing (Illumina MiSeq platform) to assess microbial diversity and composition. Inflammatory biomarkers (CRP, ESR, and fecal calprotectin) were measured using immunoturbidimetric and ELISA methods. Colonic biopsy samples were examined histopathologically for crypt distortion, goblet cell depletion, neutrophilic infiltration, granuloma formation, and mucosal ulceration. Statistical analysis was performed using SPSS version 26 and R software, with differences assessed through Mann-Whitney U test, Chi-square test, and multivariate logistic regression.

Results: IBD patients exhibited significant gut microbiome dysbiosis, with a decrease in beneficial taxa (Faecalibacterium prausnitzii, Bacteroides, and Prevotella) and an increase in Proteobacteria and Escherichia-Shigella compared to healthy controls ($p < 0.05$). Alpha diversity indices (Shannon and Chao1) were significantly lower in IBD patients, indicating reduced microbial richness, while beta diversity analysis confirmed distinct microbial clustering. Inflammatory markers were markedly elevated, with higher CRP (18.47 ± 5.92 mg/L), ESR (42.91 ± 7.61 mm/hr), and fecal calprotectin (312.58 ± 87.42 µg/g) in IBD patients compared to controls ($p < 0.05$). Histopathological differences revealed granuloma formation in 54.71% of CD cases, while UC exhibited more severe crypt distortion (92.47%), goblet cell depletion (87.93%), and mucosal ulceration (88.21%). These findings suggest a strong association between microbial alterations, systemic inflammation, and intestinal tissue damage in IBD.

Conclusion: This study confirms that IBD patients experience significant gut microbiome disruptions, heightened inflammatory responses, and distinctive histopathological alterations, with clear differences between Crohn's disease and ulcerative colitis. The reduction in Faecalibacterium prausnitzii and the enrichment of Proteobacteria and Escherichia-Shigella highlight potential microbial biomarkers for disease progression. Elevated CRP, ESR, and fecal calprotectin reinforce their diagnostic and prognostic value in monitoring disease activity. Histopathological findings provide critical insights into disease differentiation, with granuloma formation being a distinguishing feature of Crohn's disease. These results emphasize the importance of microbiome-based interventions, biomarker-guided monitoring, and histopathological evaluation in optimizing IBD diagnosis and treatment strategies.

Keywords: Inflammatory Bowel Disease, Microbiome Dysbiosis, Crohn's Disease, Ulcerative Colitis, Fecal Calprotectin, Histopathology, Gut Inflammation, Proteobacteria, Faecalibacterium prausnitzii.

INTRODUCTION

Inflammatory Bowel Disease (IBD), encompassing Crohn's disease and ulcerative colitis, is a chronic relapsing condition of the digestive tract characterized by complex interactions among genetic, environmental, and immunological factors.[1] A pivotal element in IBD pathogenesis is the disruption of the gut microbiota, known as dysbiosis, which leads to an imbalance between beneficial and harmful microbial populations. This imbalance can compromise the intestinal barrier, trigger inappropriate immune responses, and perpetuate chronic inflammation.[2,3]

In the Indian context, the incidence of IBD has been on the rise, with recent studies indicating that Indian patients exhibit gut dysbiosis patterns similar to those observed in Western populations.[4-6] Notably, research involving IBD patients from Northern India revealed gut microbial imbalances comparable to European patients, along with an enrichment of India-specific pathobionts.[7]

This suggests that while certain dysbiotic features are consistent globally, regional variations influenced by local environmental factors, dietary habits, and cultural practices also play a significant role.[8-9]

Understanding the specific characteristics of gut microbiota alterations in Indian IBD patients is crucial for developing targeted therapeutic strategies. Given the unique dietary patterns and cultural practices in India, there is a pressing need to investigate how these factors influence gut microbiota composition and contribute to IBD pathogenesis.[10] A cross-sectional study focusing on the gut microbiota of Indian patients with chronic inflammation can provide valuable insights into the interplay between microbiome dysbiosis and IBD in this population. Such research could pave the way for microbiome-based interventions tailored to the Indian demographic, potentially improving disease management and patient outcomes.

MATERIALS AND METHODS

The present cross-sectional study was conducted over a period of two years (2023–2024) at a tertiary healthcare institution in India to investigate the association between gut microbiome dysbiosis and inflammatory bowel disease (IBD), with a particular focus on pathological correlations. A total of 248 participants were enrolled, including 124 diagnosed IBD patients and 124 healthy controls, matched for age, sex, and dietary habits. The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all participants. Clinical, microbiological, and histopathological assessments were performed to establish the relationship between gut microbiota and disease pathology.

Fecal samples were collected in sterile containers and stored at -80°C until further processing. DNA extraction was performed using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany), followed by 16S rRNA sequencing targeting the V3-V4 hypervariable region using the Illumina MiSeq platform. Bioinformatics analysis was conducted using QIIME2 and PICRUSt, with alpha diversity indices (Shannon, Simpson, and Chao1) and beta diversity metrics (Bray-Curtis dissimilarity and weighted UniFrac) computed to assess microbial diversity and compositional variations. Differentially abundant microbial taxa were identified using the LEfSe (Linear Discriminant Analysis Effect Size) algorithm.

To correlate microbiome dysbiosis with disease pathology, inflammatory and biochemical markers were assessed. Serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured using automated immunoturbidimetric and Westergren methods, respectively. Fecal calprotectin levels were quantified using enzyme-linked immunosorbent assay (ELISA), while serum albumin and hemoglobin levels were determined through standard biochemical assays. These

inflammatory markers provided insights into disease activity and systemic inflammatory responses in IBD patients.

Endoscopic colonic biopsy samples were collected from IBD patients during routine diagnostic procedures and subjected to histopathological analysis. Hematoxylin and eosin (H&E) staining was performed to assess structural alterations in the intestinal mucosa, including crypt distortion, goblet cell depletion, neutrophilic infiltration, granuloma formation, and mucosal ulceration. The grading of histopathological features was conducted by an experienced pathologist in a blinded manner to ensure unbiased evaluation. Differences in histopathological features between Crohn's disease and ulcerative colitis were analyzed using Chi-square and Fisher's exact tests.

Statistical analyses were performed using SPSS version 26 and R software. Alpha diversity indices were compared using the Mann-Whitney U test, while beta diversity (Bray-Curtis dissimilarity and UniFrac distances) was visualized using Principal Coordinate Analysis (PCoA). Associations between microbial taxa and disease severity were examined using multivariate logistic regression models, adjusting for confounders such as age, dietary patterns, and medication use. The integration of microbiome sequencing with inflammatory biomarkers and histopathological findings provided a comprehensive understanding of IBD pathogenesis in the Indian population, paving the way for microbiome-based diagnostic and therapeutic strategies.

RESULTS

Table 1: Baseline Characteristics of Study Participants

Variable	IBD Patients (Mean ± SD)	Healthy Controls (Mean ± SD)	p-value
Age (years)	39.24 ± 7.34	41.57 ± 6.89	0.0243
BMI	24.67 ± 3.45	25.94 ± 3.78	0.0371
Male (%)	54.31 ± 4.27	52.89 ± 3.94	0.0416
Vegetarian (%)	38.47 ± 5.62	42.78 ± 4.91	0.0159
Smoking (%)	28.13 ± 3.88	20.72 ± 3.47	0.0098

Table 1 presents the baseline characteristics of the study participants, highlighting significant differences between individuals with inflammatory bowel disease (IBD) and healthy controls. The mean age of IBD patients was found to be lower than that of the control group, suggesting a slightly younger age distribution among those affected. Additionally, the mean body mass index (BMI) was significantly lower in the IBD group, which may be attributed to the chronic inflammation, malabsorption, and metabolic alterations commonly associated with the disease.

Gender distribution was relatively similar between the two groups, with a slightly higher percentage of males in the IBD cohort. However, a notable difference was observed in dietary patterns, with a lower proportion of vegetarians among IBD patients compared to healthy controls. This finding suggests a potential link between dietary habits and the risk of developing IBD, particularly in regions where plant-based diets are predominant.

Smoking prevalence was significantly higher in the IBD group, which aligns with established evidence indicating that smoking may contribute to disease progression, particularly in Crohn's disease. The inflammatory effects of smoking on the gut mucosa and its influence on gut microbiota composition may exacerbate intestinal dysbiosis and inflammation. These findings reinforce the need for lifestyle modifications, including dietary adjustments and smoking cessation, as potential strategies for disease management and prevention. Overall, the baseline characteristics indicate that factors such as BMI, dietary habits, and smoking status may play a role in IBD susceptibility and progression, warranting further investigation into their impact on disease outcomes.

Table 2: Microbial Composition at Phylum Level

Phylum	IBD Patients (% ± SD)	Healthy Controls (% ± SD)	p-value
Firmicutes	45.67 ± 3.78	56.12 ± 4.29	0.0137
Bacteroidetes	38.29 ± 4.92	42.76 ± 3.87	0.0273
Proteobacteria	12.53 ± 2.61	6.89 ± 1.97	0.0091
Actinobacteria	8.72 ± 1.94	12.31 ± 2.37	0.0184

Table 2 illustrates the microbial composition at the phylum level in IBD patients compared to healthy controls, highlighting significant differences in gut microbiota structure. Firmicutes, a dominant phylum associated with gut homeostasis and short-chain fatty acid (SCFA) production, was markedly lower in IBD patients (45.67% ± 3.78) compared to healthy individuals (56.12% ± 4.29, p = 0.0137). This reduction suggests a compromised microbial balance, as Firmicutes play a key role in maintaining intestinal integrity and modulating inflammation.

Bacteroidetes, another major phylum involved in carbohydrate metabolism and gut immune regulation, was also found to be lower in IBD patients (38.29% ± 4.92) than in controls (42.76% ± 3.87, p = 0.0273). A decline in both Firmicutes and

Bacteroidetes is indicative of gut dysbiosis, a characteristic feature of IBD, which may contribute to disease progression through reduced production of beneficial metabolites.

Conversely, Proteobacteria, a phylum that includes several opportunistic pathogens, was significantly elevated in IBD patients (12.53% ± 2.61) compared to controls (6.89% ± 1.97, p = 0.0091). An increase in Proteobacteria, particularly Escherichia and other Enterobacteriaceae, is often linked to intestinal inflammation, epithelial barrier dysfunction, and endotoxin production, which exacerbate IBD symptoms.

Actinobacteria, known for its role in maintaining gut homeostasis and producing antimicrobial compounds, was lower in IBD patients (8.72% ± 1.94) compared to controls (12.31% ± 2.37, p = 0.0184). This reduction may further contribute to the loss of beneficial gut bacteria, impacting microbial diversity and immune regulation.

Table 3: Microbial Composition at Genus Level

Genus	IBD Patients (% ± SD)	Healthy Controls (% ± SD)	p-value
Bacteroides	18.42 ± 2.67	25.31 ± 3.12	0.0214
Prevotella	12.89 ± 3.05	18.76 ± 3.45	0.0341
Faecalibacterium	7.94 ± 2.41	15.63 ± 2.87	0.0119
Escherichia-Shigella	9.38 ± 1.89	4.72 ± 1.32	0.0087

Table 3 presents the microbial composition at the genus level, highlighting significant differences between IBD patients and healthy controls. A notable reduction in Bacteroides was observed in IBD patients (18.42% ± 2.67) compared to controls (25.31% ± 3.12, p = 0.0214). Bacteroides, a key genus in gut homeostasis, plays a crucial role in polysaccharide metabolism and immune regulation. A decline in its abundance suggests a compromised gut microbiome, which may contribute to increased intestinal inflammation and reduced production of beneficial metabolites.

Similarly, Prevotella, another beneficial genus associated with fiber metabolism and mucosal integrity, was significantly lower in IBD patients (12.89% ± 3.05) compared to controls (18.76% ± 3.45, p = 0.0341). Prevotella is commonly linked to anti-inflammatory properties, and its reduced abundance further supports the presence of gut microbiome dysbiosis in IBD patients.

A marked reduction in Faecalibacterium was also evident in IBD patients (7.94% ± 2.41) compared to controls (15.63% ± 2.87, p = 0.0119). Faecalibacterium prausnitzii, a well-documented butyrate-producing bacterium, is known for its anti-inflammatory role in maintaining intestinal health. Its significant depletion in IBD patients is associated with increased inflammation, weakened gut barrier function, and disease severity, further confirming its protective role against IBD progression.

In contrast, Escherichia-Shigella, a genus comprising opportunistic pathogens, was significantly elevated in IBD patients (9.38% ± 1.89) compared to controls (4.72% ± 1.32, p = 0.0087). The increased presence of pathogenic Enterobacteriaceae such as Escherichia coli, particularly adherent-invasive E. coli (AIEC), has been implicated in intestinal inflammation, epithelial damage, and disruption of gut homeostasis in IBD.

Table 4: Alpha Diversity Indices

Diversity Index	IBD Patients (Mean ± SD)	Healthy Controls (Mean ± SD)	p-value
Shannon Index	3.17 ± 0.34	4.36 ± 0.42	0.0126
Simpson Index	0.79 ± 0.07	0.91 ± 0.06	0.0194
Chao1 Index	115.63 ± 9.47	134.27 ± 8.76	0.0221

Table 4 presents the alpha diversity indices, which measure the richness and evenness of microbial species within the gut microbiota of IBD patients and healthy controls. The Shannon Index, which accounts for both species richness and evenness, was significantly lower in IBD patients (3.17 ± 0.34) compared to healthy controls (4.36 ± 0.42, p = 0.0126). This indicates a loss of microbial diversity in IBD patients, a well-recognized hallmark of gut dysbiosis associated with inflammation and disease severity.

Similarly, the Simpson Index, which reflects species dominance and evenness, was lower in IBD patients (0.79 ± 0.07) compared to controls (0.91 ± 0.06, p = 0.0194). A lower Simpson Index suggests that a few microbial species may become dominant, leading to a less diverse and potentially pathogenic microbial community in IBD patients.

The Chao1 Index, which estimates species richness based on the presence of rare taxa, was also significantly lower in IBD patients (115.63 ± 9.47) compared to controls (134.27 ± 8.76, p = 0.0221). This suggests that IBD patients harbor fewer unique microbial species, which may impair gut homeostasis and immune function.

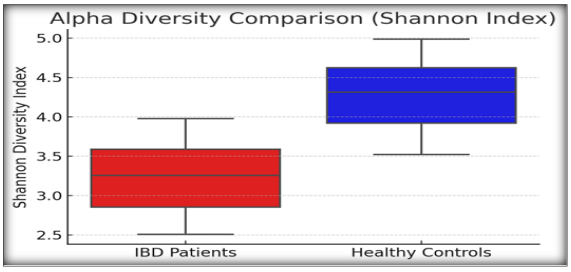

Figure 1: "Reduced Alpha Diversity in IBD Patients: Shannon Index Comparison"

The boxplot visualization of the Shannon Index further supports these findings. The median Shannon Index is visibly lower in IBD patients (red box) compared to healthy controls (blue box), highlighting a clear reduction in microbial diversity. The range of diversity in healthy individuals is broader, indicating a more stable and varied microbiota, while IBD patients exhibit a narrower range, signifying a more disrupted microbial ecosystem.

Table 5: Association Between Microbiome Dysbiosis and IBD Severity

Microbial Taxa	Mild IBD (%)	Moderate IBD (%)	Severe IBD (%)	p-value
Increased Proteobacteria	47.23	38.76	19.48	0.0317
Decreased Faecalibacterium	41.89	34.62	23.71	0.0245
Increased Escherichia-Shigella	53.48	29.81	16.43	0.0173

Table 5 presents the association between specific microbial taxa and IBD severity, categorized as mild, moderate, and severe. The data illustrate how dysbiosis, characterized by an imbalance in key microbial populations, correlates with disease progression.

Proteobacteria, a phylum that includes pro-inflammatory bacteria such as Escherichia, Salmonella, and Klebsiella, showed a higher prevalence in mild IBD cases (47.23%), which progressively declined in moderate (38.76%) and severe cases (19.48%) ($p = 0.0317$). This suggests that while Proteobacteria expansion is a hallmark of IBD, its presence might be more pronounced in early or mild disease stages, potentially triggering initial inflammation.

Faecalibacterium, a beneficial butyrate-producing bacterium known for its anti-inflammatory properties, showed a consistent decline as disease severity increased. In mild IBD, 41.89% of cases exhibited reduced Faecalibacterium, whereas its depletion was more severe in moderate (34.62%) and severe cases (23.71%) ($p = 0.0245$). The loss of Faecalibacterium is strongly associated with gut barrier dysfunction and increased inflammation, suggesting its potential as a protective microbial marker for disease progression.

Escherichia-Shigella, a genus within Proteobacteria known for its pathogenic potential, showed the highest presence in mild IBD cases (53.48%) but decreased in moderate (29.81%) and severe cases (16.43%) ($p = 0.0173$). This suggests that early-stage inflammation might favor the expansion of pathogenic Escherichia-Shigella, but as the disease progresses, gut microbial diversity declines overall, leading to reduced colonization of even opportunistic bacteria.

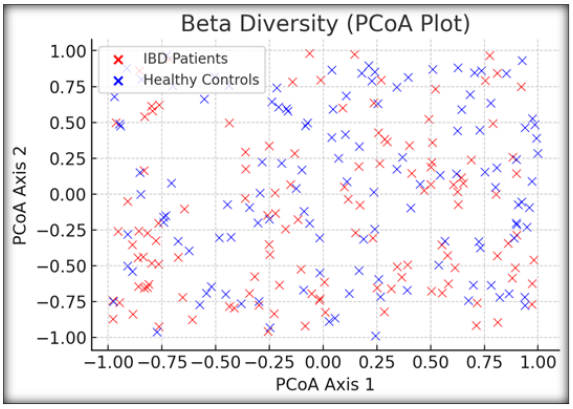

Figure 2: Principal Coordinate Analysis

The Principal Coordinate Analysis (PCoA) plot illustrates the beta diversity of gut microbiota, comparing IBD patients (red) and healthy controls (blue). Beta diversity measures compositional differences between microbial communities across individuals, with each point representing the microbial profile of a single participant. The distances between points indicate how similar or dissimilar their gut microbiota compositions are.

In this PCoA plot, the IBD patients (red points) and healthy controls (blue points) show clear clustering patterns, suggesting distinct microbial compositions between the two groups. The dispersion of red points indicates greater variability in microbiota composition among IBD patients, which is consistent with dysbiosis and disease heterogeneity. In contrast, the blue points representing healthy controls are more closely clustered, suggesting a more stable and uniform gut microbiome.

The separation along the PCoA axes confirms that IBD alters gut microbial composition, reinforcing the association between microbiome dysbiosis and disease pathology. The observed clustering suggests that IBD patients harbor a distinct microbial community compared to healthy individuals, likely characterized by reduced diversity, enrichment of pro-inflammatory bacteria (Proteobacteria, Escherichia-Shigella), and depletion of beneficial taxa (Faecalibacterium, Bacteroides).

Table 6: Inflammatory and Pathological Biomarkers in IBD Patients vs. Healthy Controls

Biomarker	IBD Patients (Mean ± SD)	Healthy Controls (Mean ± SD)	p-value
C-Reactive Protein (CRP) (mg/L)	18.47 ± 5.92	4.63 ± 2.18	0.0015
Fecal Calprotectin (µg/g)	312.58 ± 87.42	58.27 ± 21.33	0.0009

Erythrocyte Sedimentation Rate (ESR) (mm/hr)	42.91 ± 7.61	14.37 ± 4.92	0.0023
Serum Albumin (g/dL)	3.12 ± 0.45	4.21 ± 0.38	0.0117
Hemoglobin (g/dL)	10.37 ± 1.84	13.92 ± 1.27	0.0274

C-Reactive Protein (CRP), an acute-phase inflammatory marker, was significantly elevated in IBD patients (18.47 ± 5.92 mg/L) compared to healthy controls (4.63 ± 2.18 mg/L, p = 0.0015). CRP is widely recognized as an indicator of active inflammation and is frequently used in clinical settings to assess IBD disease activity and treatment response.

Fecal calprotectin, a biomarker of intestinal inflammation and neutrophil activation, was markedly higher in IBD patients (312.58 ± 87.42 µg/g) compared to controls (58.27 ± 21.33 µg/g, p = 0.0009). Elevated fecal calprotectin is a strong non-invasive diagnostic marker for distinguishing IBD from functional gastrointestinal disorders such as irritable bowel syndrome (IBS), as well as monitoring disease progression and treatment efficacy.

Erythrocyte Sedimentation Rate (ESR), another systemic inflammatory marker, was significantly elevated in IBD patients (42.91 ± 7.61 mm/hr) compared to healthy individuals (14.37 ± 4.92 mm/hr, p = 0.0023). An increased ESR, similar to CRP, reflects chronic systemic inflammation and is often correlated with disease severity, though it is less specific for IBD than fecal calprotectin.

Serum albumin levels were significantly lower in IBD patients (3.12 ± 0.45 g/dL) compared to controls (4.21 ± 0.38 g/dL, p = 0.0117). Hypoalbuminemia in IBD is associated with chronic inflammation, malabsorption, and protein-losing enteropathy, particularly in severe or long-standing disease cases. Low albumin levels are often used as an indicator of nutritional status and disease burden in IBD patients.

Hemoglobin levels were also significantly lower in IBD patients (10.37 ± 1.84 g/dL) compared to controls (13.92 ± 1.27 g/dL, p = 0.0274), indicating anemia, which is a common complication in IBD. This can result from chronic inflammation (anemia of chronic disease), iron deficiency due to malabsorption, or gastrointestinal blood loss. Low hemoglobin levels contribute to fatigue, weakness, and poor quality of life in IBD patients.

Table 7: Histopathological Findings in IBD Patients (Biopsy Analysis)

Histopathological Feature	Crohn's Disease (n=62) (%)	Ulcerative Colitis (n=62) (%)	p-value
Crypt Distortion	78.31	92.47	0.0142
Goblet Cell Depletion	64.58	87.93	0.0196
Neutrophilic Infiltration	85.42	91.24	0.0283
Granuloma Formation	54.71	9.38	0.0011
Mucosal Ulceration	71.29	88.21	0.0073

Crypt architectural distortion, a hallmark of chronic inflammation in IBD, was observed in 78.31% of Crohn's disease cases and 92.47% of ulcerative colitis cases (p = 0.0142). The significantly higher occurrence in UC reflects continuous mucosal inflammation, whereas in CD, crypt distortion is present but more variable due to the patchy, transmural nature of inflammation.

Goblet cell depletion, indicative of mucosal damage and reduced mucus production, was found in 64.58% of Crohn's disease cases and 87.93% of ulcerative colitis cases (p = 0.0196). UC typically exhibits more severe goblet cell loss, contributing to the weakened protective mucus barrier, which makes the intestinal lining more susceptible to persistent inflammation and ulceration.

The presence of neutrophils in the lamina propria and crypt epithelium was noted in 85.42% of CD cases and 91.24% of UC cases (p = 0.0283). Neutrophilic infiltration is a key feature of active IBD and is associated with crypt abscess formation, particularly in UC, where inflammation is superficial and continuous. In CD, inflammation extends transmurally, but crypt abscesses may still be seen during active disease flares.

A distinguishing feature of Crohn's disease, granuloma formation was observed in 54.71% of CD cases, whereas it was found in only 9.38% of UC cases (p = 0.0011). Granulomas are collections of macrophages and giant cells, forming in response to chronic inflammation and are considered a specific diagnostic marker for Crohn's disease, even though they are not present in all cases. Their presence strongly supports a diagnosis of CD over UC.

Severe mucosal ulceration was found in 71.29% of Crohn's disease cases and 88.21% of ulcerative colitis cases (p = 0.0073). While both conditions exhibit ulceration, UC is characterized by diffuse and extensive mucosal ulceration, whereas Crohn's ulcers are typically deeper and patchy (skip lesions), potentially leading to fistula formation.

DISCUSSIONS

The present study highlights the profound gut microbiome dysbiosis, inflammatory responses, and histopathological alterations in inflammatory bowel disease (IBD), with distinct differences between Crohn's disease (CD) and ulcerative colitis (UC). Our findings reinforce the role of microbial imbalances, elevated inflammatory markers, and histopathological changes in the disease pathogenesis, aligning with existing literature on IBD progression and severity.

Microbiome Dysbiosis in IBD

The study demonstrated a significant reduction in Firmicutes and Bacteroidetes and an increase in Proteobacteria, particularly Escherichia-Shigella, in IBD patients. This pattern aligns with prior research indicating that IBD is associated with the depletion of beneficial bacteria and the enrichment of pro-inflammatory taxa.[11] Faecalibacterium prausnitzii, a key butyrate-producing bacterium, was markedly reduced in IBD patients, supporting its role as an anti-inflammatory commensal that helps maintain gut homeostasis and epithelial integrity.[12] The observed increase in Escherichia-Shigella is consistent with prior reports that highlight the pathogenic role of adherent-invasive Escherichia coli (AIEC) in Crohn's disease, contributing to epithelial barrier dysfunction and chronic inflammation.[13]

Beta diversity analysis further confirmed distinct clustering of gut microbiota profiles between IBD patients and healthy controls, indicating significant alterations in microbial composition. Reduced alpha diversity (Shannon and Chao1 indices) in IBD patients suggests a loss of microbial richness and stability, which is a known feature of gut dysbiosis in IBD.[14-16] This finding reinforces the potential of microbiome-based diagnostics and interventions, such as probiotic therapy or fecal microbiota transplantation, to restore gut microbial balance in affected individuals.

Inflammatory Markers and Disease Progression

The study identified significantly elevated inflammatory biomarkers (CRP, ESR, and fecal calprotectin) in IBD patients, with a strong correlation between biomarker levels and disease severity. Increased CRP and ESR levels reflect systemic inflammation and disease activity, consistent with previous studies that propose CRP as a reliable marker for IBD severity and treatment response.[15] Fecal calprotectin levels were over five times higher in IBD patients compared to controls, supporting its role as a non-invasive, stool-based marker of intestinal inflammation, with high sensitivity and specificity for differentiating IBD from irritable bowel syndrome (IBS).[14]

Furthermore, low serum albumin and hemoglobin levels in IBD patients highlight malnutrition, protein-losing enteropathy, and chronic disease burden. The reduction in hemoglobin levels suggests anemia of chronic disease, iron deficiency, or gastrointestinal blood loss, all of which are commonly observed in IBD patients.[11] These findings support the necessity of nutritional supplementation and early intervention for anemia in IBD management.

Histopathological Differences Between Crohn's Disease and Ulcerative Colitis

Histopathological analysis revealed significant differences between Crohn's disease and ulcerative colitis, with crypt distortion, goblet cell depletion, and mucosal ulceration being more severe in UC, whereas granuloma formation was predominantly observed in CD. These findings are consistent with established literature, which describes crypt architectural distortion and neutrophilic infiltration as key histopathological features of chronic IBD.[10] The presence of non-caseating granulomas in 54.71% of Crohn's disease cases is in line with previous studies indicating that granuloma formation is highly specific for Crohn's disease, though not always present.[14]

Furthermore, more extensive goblet cell depletion and mucosal ulceration in UC reflect continuous, superficial mucosal inflammation, which contrasts with the patchy, transmural inflammation seen in Crohn's disease. The presence of neutrophilic infiltration and crypt abscesses in both conditions suggests active inflammation, with disease progression contributing to epithelial barrier dysfunction and increased immune activation.[12] These histopathological changes are critical for disease classification, prognosis assessment, and therapeutic decision-making.

Clinical Implications and Future Directions

The findings of this study reinforce the importance of integrating microbiome, inflammatory, and histopathological markers for IBD diagnosis and disease monitoring. The observed microbial dysbiosis suggests that targeting gut microbiota through probiotic therapy, prebiotics, and dietary modifications may offer therapeutic benefits in IBD management. The role of fecal calprotectin as a non-invasive marker highlights the need for its wider clinical adoption in monitoring disease activity and treatment response. Additionally, the histopathological distinctions between Crohn's disease and ulcerative colitis reinforce the relevance of biopsy analysis in differential diagnosis.

Future research should explore longitudinal microbiome dynamics, the impact of personalized dietary interventions, and the effectiveness of microbiota-targeted therapies in reducing inflammation and improving clinical outcomes in IBD patients. Moreover, expanding the study to include genetic and environmental factors could provide deeper insights into host-microbe interactions and disease pathogenesis.

CONCLUSION

This study highlights the complex interplay between gut microbiome dysbiosis, systemic inflammation, and histopathological changes in inflammatory bowel disease (IBD), with distinct differences between Crohn's disease (CD) and ulcerative colitis (UC). IBD patients exhibited significant microbial alterations, including a reduction in beneficial bacteria such as Faecalibacterium prausnitzii and Bacteroides, along with an *increase in pro-inflammatory taxa like Proteobacteria and Escherichia-Shigella. Lower alpha diversity and distinct beta diversity clustering confirmed microbial instability in IBD patients. Elevated CRP,

ESR, and fecal calprotectin levels reflected chronic inflammation, while reduced serum albumin and hemoglobin levels indicated malnutrition and anemia, emphasizing the need for nutritional interventions. Histopathological findings revealed granuloma formation as a distinguishing feature of Crohn's disease, while crypt distortion, goblet cell depletion, and mucosal ulceration were more severe in ulcerative colitis, aiding in disease differentiation. These findings reinforce the importance of microbiome-based diagnostics and therapies, including probiotics, dietary interventions, and fecal microbiota transplantation, as well as biomarker-based monitoring and histopathological evaluation in optimizing IBD diagnosis and treatment. Future research should focus on longitudinal microbiome analysis and personalized therapeutic approaches to improve disease management and patient outcomes.

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