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Expression of Gut Microbiota-Derived Metabolites and Their Association with Mucosal Inflammation in Ulcerative Colitis Patients

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ABSTRACT

Background

Ulcerative colitis is classified as a chronic type of inflammatory bowel disease that attacks the colon, frequently accompanied by diarrhea, abdominal pain, rectal bleeding, or both. The contribution of the gut microbiota to its pathogenesis is understood, but the exact influence of metabolites produced by bacteria on mucosa inflammation is still insufficiently investigated. To determine the level of expression of metabolites generated by gut flora and their relation to mucosal inflammation in patients suffering from ulcerative colitis.

Methods

This cross-sectional analytical study was done at Hayatabad Medical Complex, Peshawar, from January 2023 to January 2024. A total of 110 patients with confirmed ulcerative colitis were enrolled. Clinical data collection included measuring disease activity using the Mayo score, CRP, andfecal calprotectin. Stool and biopsy samples were analyzed for short-chain fatty acids, LPS, TMAO, and tryptophan derivatives. Histological grading and cytokine expression levels were also measured. Statistical methods evaluated metabolite levels and inflammation markers.

Results

Patients exhibiting high inflammatory scores showed markedly lower concentrations of anti-inflammatory metabolites such as butyrate, propionate, and indole-3-propionic acid. In contrast, LPS and TMAO were elevated and strongly correlated with increased endoscopic and histological inflammation. Increased TNF-α, IL-1β, and IL-6 concentrations were also reported in patients with high concentrations of pro-inflammatory metabolites. A distinct relationship was noted between the imbalance of microbial metabolites and the degree of inflammation present in the mucosal tissue.

Conclusion

The investigation elucidates a clear association between the disturbance of gut microbial metabolism and the mucosal inflammation associated with ulcerative colitis. The results underlined the importance of controlling microbial metabolites as markers (therapeutic targets) in the treatment and in the monitoring of the disease activity.

Keywords

Ulcerative colitis, gut microbiota, short-chain fatty acids, lipopolysaccharide, TMAO, mucosal inflammation, microbial metabolites, cytokines

INTRODUCTION

The gut microbiota refers to a diverse and complex community of microorganisms residing in the human gastrointestinal tract. These microbes play a pivotal role in digestion, immune modulation, the production of essential vitamins, and maintaining the intestinal barrier. Their metabolites act as signaling molecules that influence both local and systemic physiology. Alterations in gut microbiota composition termed dysbiosishave been implicated in a variety of diseases, including obesity, diabetes, cardiovascular disease, neurodegenerative conditions, and colorectal cancer. Emerging evidence suggests that these microorganisms and their metabolic by-products are central to the pathogenesis of numerous chronic inflammatory and autoimmune diseases [1-3].

Their involvement in inflammatory bowel diseases such as ulcerative colitis (UC) is of significant interest within this broad context.Ulcerative colitis is a form of chronic inflammatory disease of the colon (and the rectum) that manifests with recurrent abdominal discomfort, diarrhea, and rectal hemorrhage. Though several factors may predispose someone to the condition, the causa is still elusive. It is thought that some genetic vulnerability, alterations in immune response, specific environmental factors, and imbalances within the microflora are contributory. Lately, the role of intestinal bacteria and their metabolites in controlling inflammation has become increasingly popular in scientific discourse

The human microbiome is central to processes such as digestion, nutrient absorption, immune equilibrium, and the upkeep of mucosal surfaces. It is composed of trillions of microorganisms[4, 5]. These microorganisms profoundly impact the health of the intestines since they generate various metabolites, including SCFAs, bile acid derivatives, and amenable acids. Patients suffering from ulcerative colitis usually suffer from microbial community imbalances, or dysbiosis which leads to significant loss of beneficial metabolites and a surge of harmful ones [6].

As for the primary metabolites, butyrate, propionate, and indole-3-propionic acid are known to have notable anti-inflammatory effects which aid in epithelial healing and immune tolerance[7]. Conversely, compounds like lipopolysaccharides (LPS) and trimethylamine N-oxide (TMAO) are believed to activate immune responses and exacerbate inflammation. It is proposed that the interaction between these microbial substances and the immune system of the intestines is a significant factor for the destruction of the mucosa in the case of ulcerative colitis. Such interactions are considered important factors regarding the deep destruction of the mucosa during the ulcerative colitis course [8].

Gaining knowledge about the expression dynamics of these metabolites in relation to clinical and histological indices of inflammation may provide further understanding of the disease pathways as well as possible treatment avenues. The aim of this study was to measure the metabolites of the gut microbiota in patients with ulcerative colitis and assess their correlation with inflammation of the mucosa. This particular investigation works on the premise that looking at the function of the microbiota rather than its structure may explain the relationship between microbial functions and the progression of the disease.

METHODOLOGY

This was a cross-sectional analytical study that was conducted for one year, from January 2023 to January 2024, at the Department of Gastroenterology, Hayatabad Medical Complex, Peshawar. The hospital is a key referral hospital in the area, providing advanced

gastroenterological diagnostic and therapeutic services including management of inflammatory bowel diseases like ulcerative colitis (UC). The study was cleared through the Institutional Review Board (IRB) of Hayatabad Medical Complex, Peshawar. All participants provided written informed consent prior to participating in the study. Their identity as patients was protected, and all of the information gathered was entirely for the study purpose.

The study included patients with a confirmed diagnosis of ulcerative colitis, based on clinical symptoms, endoscopic findings, and histopathological confirmation. Patients were enrolled consecutively from the outpatient and inpatient departments during their routine evaluation and follow-up visits.

Inclusion Criteria

- Adults aged 18 years or older
- Diagnosed with ulcerative colitis (any disease extent)
- Willing to provide informed consent
- Able to provide stool samples and undergo colonoscopy with biopsy

Exclusion Criteria

- Patients with Crohn's disease or indeterminate colitis
- Antibiotic use within the past 6 weeks
- Current or recent probiotic or prebiotic therapy
- History of colorectal cancer or recent major surgery
- Co-existing autoimmune, hepatic, or systemic infections

A total of 110 patients were included, based on purposive sampling. The sample size was calculated considering previous literature on SCFA levels and mucosal inflammation in UC, with a confidence level of 95% and 80% power to detect a meaningful association.

After obtaining informed consent, demographic data (age, gender, BMI, smoking status, disease duration) and clinical history (medications, disease extent, flare frequency) were recorded using a structured proforma. Disease activity was assessed through Mayo score, and inflammation was evaluated using CRP and fecal calprotectin levels.

Stool samples were collected in sterile containers and stored at −80°C until analysis. Gas chromatography-mass spectrometry (GC-MS) was used to quantify short-chain fatty acids (SCFAs)—specifically acetate, propionate, and butyrate. High-performance liquid chromatography (HPLC) and ELISA kits were used to measure levels of indole-3-propionic acid, trimethylamine N-oxide (TMAO), and lipopolysaccharide (LPS).

Colonoscopy was performed in all patients to assess mucosal appearance. The Mayo endoscopic subscore was used to classify inflammation severity. Biopsy samples were taken from inflamed and non-inflamed segments and sent for histological grading using the Nancy Index. Cytokine expression (TNF-α, IL-1β, IL-6) was assessed using qPCR and ELISA from mucosal biopsy samples.

All data were analyzed using SPSS version 25. Continuous variables were reported as means with standard deviations, while categorical variables were expressed in frequencies and percentages. The independent t-test or Mann-Whitney U test was applied for comparing metabolite levels based on inflammation status. Chi-square test was used for categorical comparisons. A p-value ≤ 0.05 was considered statistically significant.

RESULT

In this study, the majority of patients with ulcerative colitis were middle-aged adults, with a slightly higher representation of females. The average BMI was within the overweight range, though not statistically linked with inflammation levels. However, smoking emerged as a significant risk factor, suggesting its possible contribution to worsening mucosal inflammation. The average duration of disease and biologic medication use did not show a statistically significant association but helped describe the baseline clinical status of patients.

Table 1: Demographic and Clinical Characteristics of Participants (n = 110)

Variable	Frequency (%) or Mean ± SD	p-value
Age (years)	38.4 ± 12.3	–
Gender (Male/Female)	48 (43.6%) / 62 (56.4%)	0.41
BMI (kg/m²)	25.8 ± 3.6	0.12
Smoking Status (Yes/No)	36 (32.7%) / 74 (67.3%)	0.03*
Duration of UC (years)	5.1 ± 2.7	0.09
Medication Use (Biologics)	45 (40.9%)	0.18

Ulcerative colitis severity was assessed using clinical indicators such as disease extent, flare-up frequency, and lab markers. Patients with pancolitis had significantly more mucosal inflammation compared to those with localized disease. Elevated disease activity scores, CRP levels, and fecal calprotectin were all significantly associated with higher inflammation, supporting their reliability as clinical markers. Frequent flare-ups also correlated with more severe disease, highlighting the impact of disease recurrence on mucosal damage.

Table 2: Clinical Disease Activity and Inflammatory Markers

Variable	Mean ± SD or Frequency (%)	p-value
Disease extent: Pancolitis	52 (47.3%)	0.04*
Mayo score (disease activity)	6.2 ± 1.9	<0.001*
Flare-ups/year	3.4 ± 1.1	0.02*
CRP (mg/L)	18.6 ± 7.2	<0.001*
Fecal Calprotectin (µg/g)	423.5 ± 108.9	<0.001*

Gut-derived metabolites showed strong associations with inflammation. Levels of beneficial short-chain fatty acids, particularly butyrate and propionate, were significantly reduced in patients with high inflammatory scores. On the other hand, harmful metabolites such as lipopolysaccharides and TMAO were elevated, indicating microbial dysbiosis. These findings suggest that changes in microbial metabolite profiles play a direct role in modulating mucosal inflammation in UC.

Table 3: Gut Microbiota-Derived Metabolite Levels

Metabolite	Mean ± SD (µmol/g or ng/mL)	p-value
Acetate	35.2 ± 5.4	0.07
Propionate	15.6 ± 3.2	0.04*
Butyrate	9.3 ± 2.8	0.02*
Indole-3-propionic acid	2.1 ± 0.6	0.03*
Lipopolysaccharide (LPS)	12.4 ± 4.1	<0.001*
TMAO	6.7 ± 1.3	0.01*

Patients with elevated mucosal inflammation had significantly higher histological severity and cytokine expression levels. Tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 were markedly increased in inflamed tissue, confirming an active immune response. The severity of the disease was also strongly associated with endoscopic scores and the presence of ulcers. These observations further strengthen the relationship between metabolites from microbiota and local immune dysregulation afflicting the colon.

Table 4: Histological and Cytokine Markers of Mucosal Inflammation

Variable	Mean ± SD or Frequency (%)	p-value
Histology (Nancy Score ≥3)	62 (56.4%)	0.01*
TNF-α expression (pg/mg)	43.6 ± 9.1	<0.001*
IL-1β (pg/mg)	27.8 ± 6.7	0.002*
IL-6 (pg/mg)	35.1 ± 7.4	<0.001*
Endoscopic Score ≥2	66 (60%)	0.001*
Presence of ulcers/erosions	71 (64.5%)	0.008*

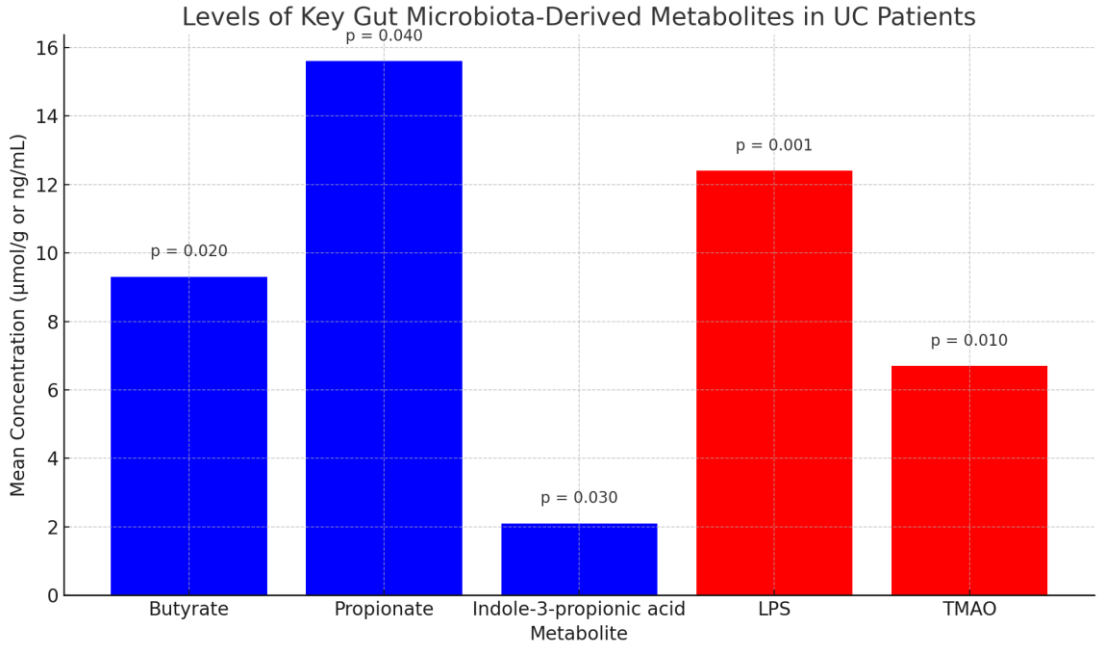

Figure 1
Reduced levels of butyrate, propionate, and indole-3-propionic acid among UC patients indicate a relative decrease in protective metabolites, as shown in the graph. Meanwhile, heightened LPS and TMAO levels indicate greater pro-inflammatory activity. These alterations depict changes in the composition of the gut microbiota which additionnally fuels mucosal inflammation.

DISCUSSION

This research analyzed the association linking gut microbial metabolites and mucosal inflammation in patients suffering from ulcerative colitis. The results underline an important change in the gut's metabolic profile, or ‘metabolome’, in individuals with active disease, characterized by diminished levels of anti-inflammatory metabolites and heightened levels of

inflammatory mediators. These findings agree with the accumulating evidence indicating that the microbiota and its metabolic by-products are instrumental in regulating intestinal immune functions [9-11].

Importantly, this study reinforces the notion that gut microbiota arerelevant in UC and play a critical role in other disease states. Dysbiosis and altered microbial metabolites have been linked to metabolic syndrome, cardiovascular diseases via TMAO elevation, neurodegenerative diseases through tryptophan pathway disturbances, and even mood disorders due to the gut-brain axis. Hence, understanding microbiota function in UC may offer insights that are translatable to a broader spectrum of disorders.

The patients with more severe inflammation httcally and endoscopically had lower levels of SCFAs, especially butyrate and propionate. Important SCFAs are produced during the fermentation of prebiotics by the gut microbiota, and SCFAs help maintain the epithelial barrier and local immunity processes. Other studies follow the same pattern. SCFAs are known to diminish some pro-inflammatory cytokines as well as assist in maintaining the integrity of epithelial tissues in cases of inflammatory bowel disease [12-14]. The depletion observed in this study's subjects likely indicates a reduction of alternative fermentation processes in patients with UC due to reduced gut bacteria.

Conversely, the patients with higher inflammation severity scores also had increased lipopolysaccharide (LPS) and trimethylamine N-oxide (TMAO) levels. These metabolites are known to induce immune system activation as well as inflammation both systematically and locally. LPS, for example, is a component of the outer membrane of Gram-negative bacteria and it can trigger toll-like receptors resulting in the production of various cytokines such as TNF- and IL-6. Our results corroborated other studies that reported strong associations of LPS with inflammatory responses in the colonic tissues of IBD patients[15-17]. While TMAO is more often investigated in relation to cardiovascular diseases, it has also been associated with gut microbiome imbalance and epithelial cell stress, thereby complicating its role in UC pathogenesis.

In our study, inflammatory cytokines such as TNF-α, IL-1β, and IL-6, that are known to be associated with the inflammation processes in UC, correspond with the system and tissue-level disease severity. Apart from that, these cytokines are also the target of numerous biologic treatments. Moreover, the association with the microbial metabolite concentration adds further evidence that the shift in gut flora is not merely a consequence of inflammation, but may participate in sustaining it" [18-20].

An important implication of these findings is the potential role of microbiota-targeted therapies in UC management. Interventions such as prebiotics, probiotics, dietary fiber supplementation, and fecal microbiota transplantation may help restore beneficial metabolite levels and suppress harmful ones, thereby modulating inflammation. However, further longitudinal and interventional studies are needed to establish causal relationships and therapeutic efficacy.

This study also highlights the complexity of host-microbe interactions in UC. While microbial composition has been the focus of much research, metabolomic profiling adds an important layer of functional insight. By understanding what the microbiota is producing, rather than just who is there, we gain a clearer picture of disease mechanisms.

There were a few limitations to this study. Being cross-sectional, it does not establish causality. Additionally, external factors such as diet, lifestyle, and unreported medication use

could have influenced metabolite levels. Despite these, the study offers strong evidence linking gut microbial metabolism with mucosal inflammation in UC.

CONCLUSION

The study revealed that ulcerative colitis patients with active inflammation exhibit reduced levels of protective metabolites like butyrate and increased levels of inflammatory markers such as LPS and TMAO. These changes were closely associated with higher histological and endoscopic inflammation scores and elevated cytokine levels.

These findings highlight the importance of gut microbial metabolites in the pathogenesis and monitoring of ulcerative colitis and echo their broader relevance in other systemic diseases. The gut microbiota and its metabolites emerge as promising diagnostic and therapeutic targets across various inflammatory and metabolic conditions.

The findings suggest that microbial metabolite profiling could be a valuable tool in assessing disease activity and may open new avenues for targeted therapies that restore microbial balance and reduce inflammation. Further research is needed to confirm these associations and explore their therapeutic potential.

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