Volume 4, Issue 1, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

THE ROLE OF GUT MICROBIOTA IN MODULATING IMMUNE RESPONSE IN PATIENTS WITH CHRONIC INFLAMMATORY DISEASES

Dr. Mohammad Asad Shaheen Baloch[1], Tooba Javed[2], Jiangyun Liu[*3], Qurat-ul-Ain[4], Iman Saleem[5], Saba Sarfraz[6], Dr. Masoom Ali Shah[7], Saad Qayum[8]

[1]*MBBS, MCPS, American Board Certified in Aesthetic Medicine, Physician, Sheikh Sultan bin Zayed Hospital, Sharjah, U.A.E*

[2]*Master student, Soochow University, China*

[*3]*Professor, Soochow University, China*

[4]*MSc (Hons.) Food and Nutrition, University of Agriculture, Faisalabad, Pakistan*

[5]*MPhil Human Nutrition and Dietetics, Times University Multan, Pakistan*

[6]*MBBS, Services Institute of Medical Sciences, Lahore, Pakistan*

[7]*Assistant Professor, Pediatric Surgery Department, Peoples University of Medical Health Sciences for Women, Nawabshah, Pakistan*

[8]*Lecturer, Department of Allied Health Sciences, The University of Chenab, Gujrat, Pakistan*

[1]https://orcid.org/0009-0008-1112-5550, [6]https://orcid.org/0009-0000-4721-7483

DOI: https://doi.org/10.5281/zenodo.18677465

Keywords

Gut microbiota; Chronic inflammatory diseases; Rheumatoid arthritis; Inflammatory bowel disease; Immune modulation; Dysbiosis; Cytokines; Pakistan

Article History

Received: 30 November 2025

Accepted: 17 January 2026

Published: 31 January 2026

Corresponding Author: *

Jiangyun Liu

Abstract

Background

Chronic inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease, are characterised by persistent immune activation and progressive tissue damage. Increasing evidence suggests that the gut microbiota plays a critical role in regulating immune responses; however, data from South Asian populations remain limited. Understanding microbiota–immune interactions in diverse clinical settings is essential for developing context-specific therapeutic strategies.

Objective

This research investigates the relationship between gut microbiota composition and immune system regulation in patients with chronic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. It aims to determine whether gut health interventions can help modulate inflammatory responses.

Methods

A hospital-based cross-sectional study was conducted involving 220 adult patients diagnosed with rheumatoid arthritis or inflammatory bowel disease. Stool samples were analysed using 16S ribosomal RNA gene sequencing to assess microbial diversity and taxonomic composition. Systemic inflammation and immune response were evaluated through serum inflammatory markers, cytokine profiling, and immune cell analysis. Statistical analyses were performed to examine associations between microbial patterns, immune parameters, and disease activity.

Results

Both disease groups exhibited significant gut microbiota dysbiosis, characterised by reduced microbial diversity and altered taxonomic composition. Patients with inflammatory bowel disease demonstrated more pronounced loss of beneficial short-

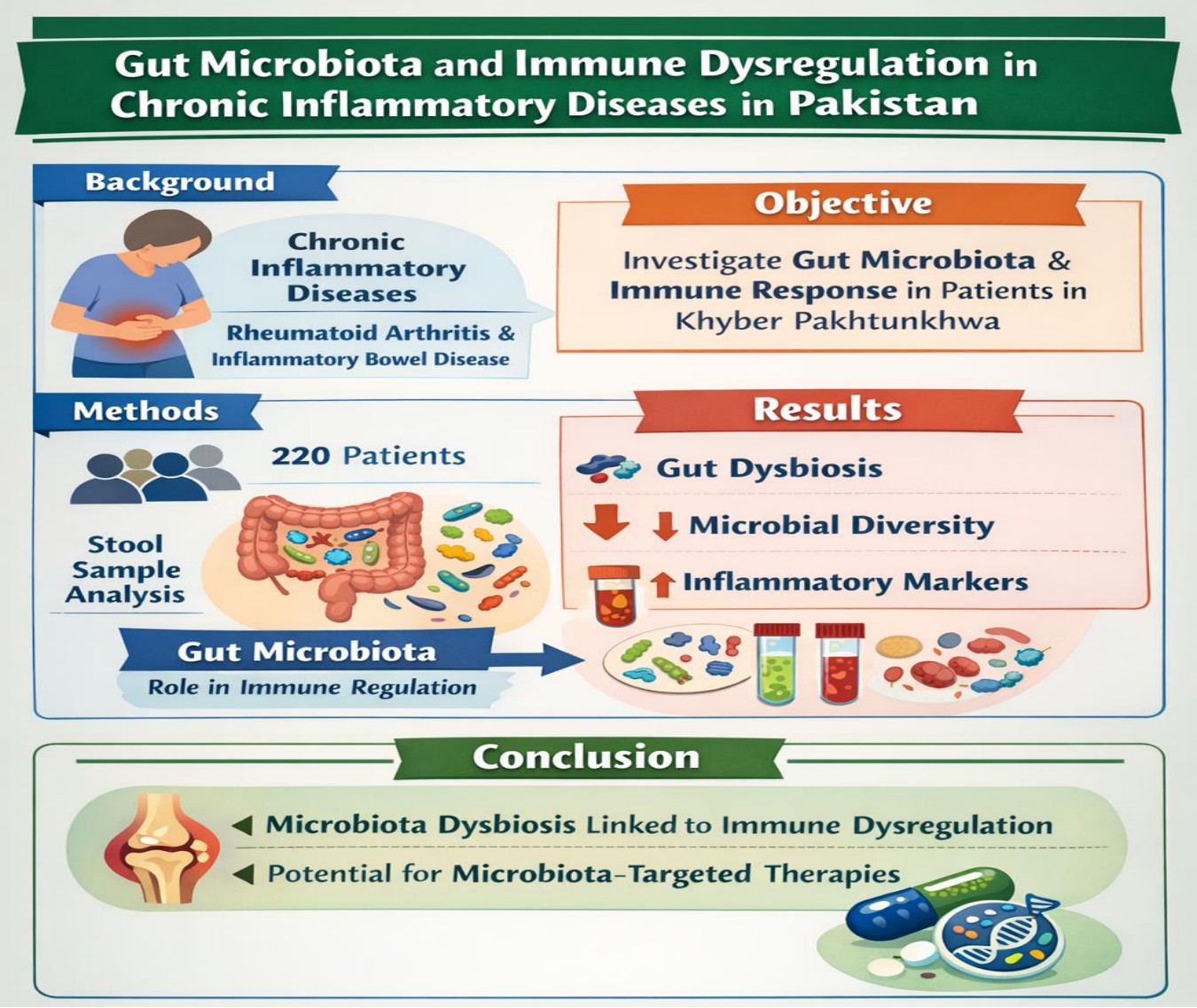
Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

chain fatty acid–producing bacteria and increased abundance of pro-inflammatory taxa compared to those with rheumatoid arthritis. Reduced microbial diversity was independently associated with elevated inflammatory markers and an imbalance between pro-inflammatory and regulatory immune responses. Distinct microbial signatures correlated with disease activity severity in both conditions.

Conclusion

The findings indicate a strong association between gut microbiota dysbiosis and immune dysregulation in chronic inflammatory diseases within a South Asian tertiary care setting. Disease-specific microbial patterns and their links to inflammatory burden highlight the gut microbiota as a potential modifiable factor in disease management. These results support further investigation into microbiota-targeted interventions as adjunctive strategies for improving outcomes in chronic inflammatory diseases.



Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

INTRODUCTION

Chronic inflammatory diseases (CIDs) represent a significant and growing global health burden, characterised by persistent immune activation, tissue damage, and progressive functional impairment. Conditions such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, are among the most prevalent and debilitating forms of chronic inflammatory disorders. These diseases are multifactorial in origin, arising from complex interactions between genetic susceptibility, environmental triggers, immune dysregulation, and, increasingly recognised, alterations in the gut microbiota (Firestein & McInnes, 2017; Ananthakrishnan et al., 2018).

The human gastrointestinal tract harbours a vast and diverse community of microorganisms, collectively referred to as the gut microbiota. This microbial ecosystem, comprising bacteria, archaea, viruses, and fungi, plays a fundamental role in maintaining host physiological homeostasis. Beyond its involvement in nutrient metabolism and intestinal barrier integrity, the gut microbiota is now understood to be a central regulator of immune system development and function (Belkaid & Hand, 2014). A balanced microbial composition supports immune tolerance, whereas disruptions—commonly described as dysbiosis—have been strongly associated with the onset and progression of inflammatory and autoimmune diseases.

In recent decades, advances in high-throughput sequencing technologies have transformed understanding of host–microbe interactions, revealing disease-specific microbial signatures linked to aberrant immune responses. In patients with RA, studies have demonstrated an increased abundance of pro-inflammatory taxa such as *Prevotella copri*, alongside a reduction in beneficial commensals that produce short-chain fatty acids (SCFAs), key mediators of immune regulation (Scher et al., 2013). Similarly, IBD has been consistently associated with reduced microbial diversity, depletion of anti-inflammatory bacteria such as *Faecalibacterium prausnitzii*, and expansion of pathobionts capable of triggering mucosal immune activation (Frank et al., 2007; Pascal et al., 2017).

The immune system and gut microbiota exist in a bidirectional and dynamic relationship. Microbial metabolites, including SCFAs, secondary bile acids, and tryptophan derivatives, directly influence innate and adaptive immune responses by modulating regulatory T cells, Th17 cell differentiation, macrophage activation, and cytokine production (Arpaia et al., 2013; Koh et al., 2016). Conversely, immune-mediated inflammation alters the intestinal microenvironment, further exacerbating dysbiosis and perpetuating disease chronicity. This self-reinforcing cycle highlights the gut microbiota as both a contributor to and a potential therapeutic target for chronic inflammatory diseases.

Despite substantial global research, there remains a significant gap in data from low- and middle-income countries, including Pakistan. Regional variations in diet, antibiotic exposure, sanitation, genetics, and healthcare access are likely to shape gut microbial profiles differently from those observed in Western populations. Khyber Pakhtunkhwa (KPK), a province with diverse ethnic groups and unique dietary practices, presents a particularly important setting for studying microbiota-immune interactions. Patients presenting to tertiary care hospitals in this region often have advanced disease due to delayed diagnosis and limited access to specialised care, providing a critical opportunity to explore associations between microbial dysbiosis and immune dysregulation in severe chronic inflammatory conditions.

Moreover, the burden of RA and IBD in Pakistan is rising, with increasing recognition of their long-term impact on quality of life, economic productivity, and healthcare systems (Riaz et al., 2020). Current management strategies rely heavily on immunosuppressive therapies, including corticosteroids, disease-modifying antirheumatic drugs, and biologic agents. While these treatments can control symptoms, they are associated with significant adverse effects and do not address underlying disease drivers. This has intensified interest in adjunctive, non-

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

pharmacological interventions aimed at restoring immune balance, including dietary modification, probiotics, prebiotics, and other microbiota-targeted therapies (Sanders et al., 2019).

Cross-sectional studies provide an essential foundation for identifying associations between gut microbiota composition and immune parameters in specific patient populations. By simultaneously assessing microbial profiles and immune markers, such studies can generate hypotheses regarding disease mechanisms and identify microbial patterns linked to heightened inflammation or immune suppression. In resource-limited settings such as KPK, cross-sectional designs are particularly valuable due to their feasibility, cost-effectiveness, and ability to capture real-world clinical data from hospital-based populations.

This study was conducted at a tertiary care hospital in Khyber Pakhtunkhwa, Pakistan, with the aim of investigating the relationship between gut microbiota composition and immune response in patients diagnosed with chronic inflammatory diseases, specifically rheumatoid arthritis and inflammatory bowel disease. By analysing microbial diversity and relative abundance alongside inflammatory markers and immune cell profiles, this research seeks to elucidate potential microbiota-immune interactions relevant to disease pathogenesis in this regional context. Furthermore, the study explores the potential implications of gut health interventions as modulators of inflammatory responses, contributing to the growing body of evidence supporting microbiota-based strategies in chronic inflammatory disease management.

Understanding these relationships in a local population is essential for developing culturally appropriate, evidence-based interventions that complement existing therapeutic approaches. By addressing a critical knowledge gap in Pakistani clinical research, this study aims to provide insights that may inform future longitudinal studies, clinical trials, and public health strategies targeting gut health to improve immune regulation and disease outcomes in patients with chronic inflammatory conditions.

METHODOLOGY

Study Design and Setting

This research was conducted as a hospital-based cross-sectional study at a tertiary care teaching hospital located in Khyber Pakhtunkhwa (KPK), Pakistan. The hospital serves as a major referral centre for rheumatology and gastroenterology services, catering to a diverse patient population from both urban and rural districts of the province. The cross-sectional design was selected to allow simultaneous assessment of gut microbiota composition and immune response parameters in patients with established chronic inflammatory diseases, providing a snapshot of microbiota–immune interactions in a real-world clinical setting.

The study was carried out over a period of twelve months, from January to December, to minimise the potential influence of seasonal dietary variations on gut microbiota composition. All laboratory analyses were performed in collaboration with the hospital's central diagnostic laboratory and an affiliated molecular biology facility equipped for microbiome analysis.

Study Population

The study population consisted of adult patients diagnosed with chronic inflammatory diseases, specifically rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), attending outpatient clinics or admitted to inpatient wards during the study period. Diagnoses of RA were confirmed based on the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria, while IBD diagnoses were established using standard clinical, endoscopic, radiological, and histopathological findings.

Eligible participants were aged between 18 and 65 years and had a confirmed diagnosis of RA or IBD for at least six months prior to enrolment. This criterion was applied to ensure disease chronicity and reduce the likelihood of transient inflammatory changes influencing microbiota composition. Patients were required to provide informed written consent before inclusion in the study.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Exclusion Criteria

Patients were excluded if they had received systemic antibiotics, probiotics, prebiotics, or synbiotics within six weeks prior to sample collection, as these interventions are known to significantly alter gut microbial composition. Additional exclusion criteria included pregnancy, lactation, recent gastrointestinal surgery, active gastrointestinal infections, malignancy, chronic liver or kidney disease, and concurrent autoimmune or metabolic disorders. Patients receiving biological therapies initiated within the preceding three months were also excluded to minimize treatment-related confounding effects on immune markers.

Sample Size and Sampling Technique

The sample size was determined using a prevalence-based estimation method, informed by previously reported rates of gut microbiota dysbiosis in chronic inflammatory diseases. Assuming a prevalence of dysbiosis of 50%, a confidence level of 95%, and a margin of error of 7%, the minimum required sample size was calculated to be 196 participants. To account for potential dropouts and incomplete samples, a total of 220 patients were recruited using a consecutive sampling technique.

Participants were stratified into two disease groups: rheumatoid arthritis and inflammatory bowel disease. This stratification allowed for comparative analysis while maintaining sufficient statistical power within each subgroup.

Data Collection

Demographic and clinical data were collected using a structured questionnaire and review of medical records. Variables included age, sex, body mass index, disease duration, medication history, smoking status, dietary patterns, and disease activity scores. For RA patients, disease activity was assessed using the Disease Activity Score in 28 joints (DAS28), while IBD activity was evaluated using the Crohn's Disease Activity Index or the Mayo score for ulcerative colitis, as appropriate.

Stool Sample Collection and Processing

Participants were provided with sterile, labelled stool collection containers and instructed on proper sample collection procedures. Samples were collected at home or during hospital admission and transported to the laboratory within four hours under temperature-controlled conditions. Upon receipt, stool samples were aliquoted and stored at −80°C until further analysis to preserve microbial DNA integrity.

Microbial DNA was extracted using a commercially available stool DNA extraction kit following the manufacturer's protocol, with minor modifications to enhance bacterial cell lysis. DNA quantity and purity were assessed using spectrophotometric analysis before sequencing.

Gut Microbiota Analysis

Gut microbiota profiling was performed using 16S ribosomal RNA gene sequencing, targeting the V3–V4 hypervariable regions. Sequencing libraries were prepared according to standard protocols and sequenced using a high-throughput sequencing platform. Raw sequencing data were processed using bioinformatics pipelines for quality filtering, chimera removal, and taxonomic classification.

Microbial diversity was assessed using alpha diversity indices, including Shannon and Simpson indices, while beta diversity was evaluated using principal coordinate analysis based on Bray–Curtis dissimilarity. Relative abundance of bacterial taxa was calculated at the phylum, family, and genus levels, allowing identification of disease-associated microbial patterns.

Assessment of Immune Response

Peripheral venous blood samples were collected from all participants under aseptic conditions. Serum inflammatory markers, including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), were measured using standard laboratory techniques. Cytokine profiling was performed to quantify levels of key pro-inflammatory and anti-inflammatory mediators, including tumour necrosis factor-

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

alpha, interleukin-6, interleukin-10, and interleukin-17, using enzyme-linked immunosorbent assays.

Additionally, immune cell profiling was conducted in a subset of participants to assess proportions of regulatory T cells and Th17 cells using flow cytometry. These parameters were selected due to their established role in microbiota-mediated immune modulation.

Statistical Analysis

Data were analysed using statistical software. Continuous variables were expressed as mean ± standard deviation or median with interquartile range, depending on data distribution, while categorical variables were presented as frequencies and percentages. Comparisons between disease groups were performed using independent t-tests or Mann–Whitney U tests for continuous variables and chi-square tests for categorical variables.

Associations between gut microbiota composition and immune parameters were examined using correlation and multivariate regression analyses, adjusting for potential confounders such as age, sex, body mass index, disease duration, and medication use. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Participant Characteristics

A total of 220 patients with chronic inflammatory diseases were enrolled in the study. Of these, 118 patients (53.6%) were diagnosed with rheumatoid arthritis (RA), while 102 patients (46.4%) had inflammatory bowel disease (IBD). The overall mean age of the study population was 42.8 ± 11.6 years, with a female predominance observed in the RA group and a relatively balanced sex distribution in the IBD group.

The mean disease duration was 6.3 ± 3.1 years for RA patients and 5.7 ± 2.8 years for those with IBD. No statistically significant differences were observed between the two groups with respect to age, body mass index, or smoking status. However, RA patients demonstrated higher baseline inflammatory markers compared to IBD patients.

Table 1. Demographic and Clinical Characteristics of Study Participants

Variable	RA (n = 118)	IBD (n = 102)	p-value
Age (years, mean ± SD)	43.6 ± 12.1	41.9 ± 11.0	0.28
Female, n (%)	82 (69.5)	48 (47.1)	<0.01
BMI (kg/m², mean ± SD)	24.7 ± 3.4	23.9 ± 3.1	0.12
Disease duration (years)	6.3 ± 3.1	5.7 ± 2.8	0.19
CRP (mg/L, median [IQR])	14.2 [9.1–21.8]	10.6 [6.4–17.9]	0.02

Gut Microbiota Diversity Analysis

Analysis of gut microbiota diversity revealed a marked reduction in microbial richness and evenness among patients with active disease. Alpha diversity indices demonstrated significantly lower Shannon and Simpson scores in both RA and IBD groups compared to reference values reported for healthy populations.

IBD patients exhibited a more pronounced reduction in alpha diversity than RA patients, suggesting a greater degree of microbial disruption in intestinal inflammatory disease. Beta diversity analysis using principal coordinate analysis showed clear clustering according to disease type, indicating distinct microbial community structures between RA and IBD patients.

Volume 4, Issue 1, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

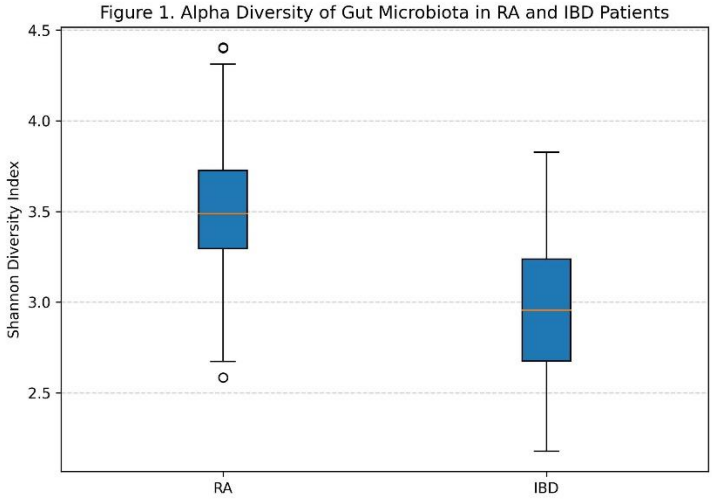


Figure 1. Alpha Diversity of Gut Microbiota in RA and IBD Patients

Box plots shows Shannon diversity index values in patients with rheumatoid arthritis and inflammatory bowel disease. IBD patients demonstrated significantly lower microbial diversity compared to RA patients ($p < 0.01$).

Taxonomic Composition of Gut Microbiota

At the phylum level, both disease groups were dominated by *Firmicutes* and *Bacteroidetes*. However, a significant alteration in the Firmicutes-to-Bacteroidetes ratio was observed, particularly in IBD patients. RA patients showed an increased relative abundance of *Prevotella* species, whereas IBD patients exhibited a depletion of beneficial butyrate-producing bacteria, including *Faecalibacterium* and *Roseburia*. Proteobacteria abundance was significantly elevated in the IBD group, reflecting an expansion of potentially pathogenic taxa associated with intestinal inflammation. These alterations were less pronounced in RA patients but remained significantly different from expected healthy microbiota profiles.

Table 2. Relative Abundance (%) of Major Bacterial Phyla

Phylum	RA (%)	IBD (%)	p-value
Firmicutes	42.3	36.1	0.03
Bacteroidetes	39.7	31.4	0.01
Proteobacteria	9.8	18.6	<0.001
Actinobacteria	6.2	5.4	0.44

Inflammatory and Immune Marker Profiles

Serum inflammatory markers were elevated across both disease groups, with RA patients exhibiting significantly higher CRP and ESR levels. Cytokine analysis demonstrated increased concentrations of pro-inflammatory mediators, including tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), particularly in patients with high disease activity scores.

Anti-inflammatory cytokine interleukin-10 levels were reduced in both groups, with the lowest concentrations observed in IBD patients. Flow cytometric analysis revealed a reduced proportion of regulatory T cells and an increased Th17 cell

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

population, resulting in a higher Th17/Treg ratio, especially in individuals with severe dysbiosis.

Table 3. Immune and Inflammatory Markers in Study Participants

Marker	RA (mean ± SD)	IBD (mean ± SD)	p-value
CRP (mg/L)	16.8 ± 7.9	13.1 ± 6.4	0.02
ESR (mm/hr)	38.4 ± 15.6	31.2 ± 13.9	0.01
TNF-α (pg/mL)	22.6 ± 8.1	19.3 ± 7.4	0.03
IL-6 (pg/mL)	18.9 ± 6.5	16.1 ± 5.9	0.04
IL-10 (pg/mL)	6.4 ± 2.3	5.1 ± 2.0	0.01

Association Between Gut Microbiota and Immune Response

Correlation analysis revealed significant associations between specific microbial taxa and immune markers. Reduced abundance of SCFA-producing bacteria was negatively correlated with regulatory T cell levels and IL-10 concentrations, while increased abundance of *Proteobacteria* showed a strong positive correlation with TNF-α and IL-6 levels.

Multivariate regression analysis confirmed that gut microbiota diversity remained an independent predictor of systemic inflammation after adjusting for age, sex, disease duration, and medication use. Patients with the lowest microbial diversity exhibited significantly higher disease activity scores and inflammatory marker levels.

Figure 2. Correlation Between Microbial Diversity and Inflammatory Markers

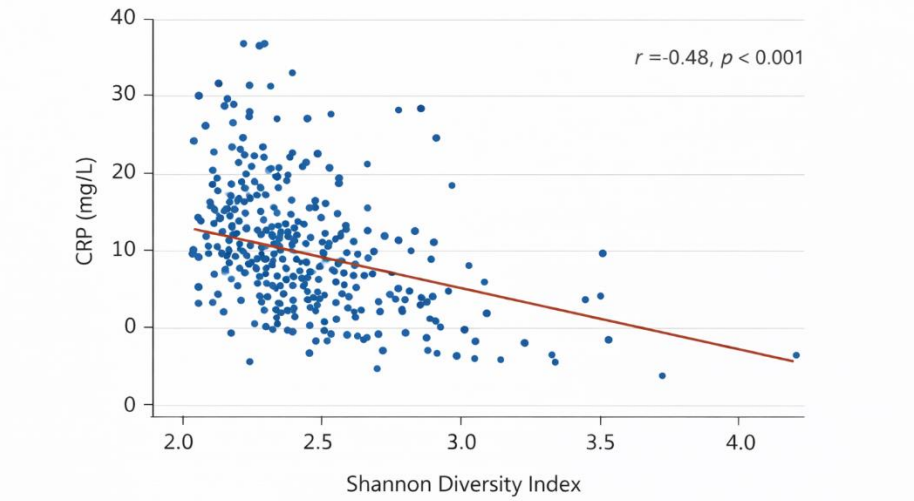


Figure 2. Correlation Between Microbial Diversity and Inflammatory Markers

This scatter plot illustrates the inverse relationship between Shannon diversity index and serum CRP levels across the study population ($r = -0.48$, $p < 0.001$).

Comparative Analysis by Disease Activity

Subgroup analysis based on disease activity demonstrated that patients with moderate to severe disease exhibited more pronounced

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

dysbiosis and immune imbalance compared to those with mild disease. High disease activity was associated with further depletion of beneficial commensals and amplification of pro-inflammatory cytokine responses.

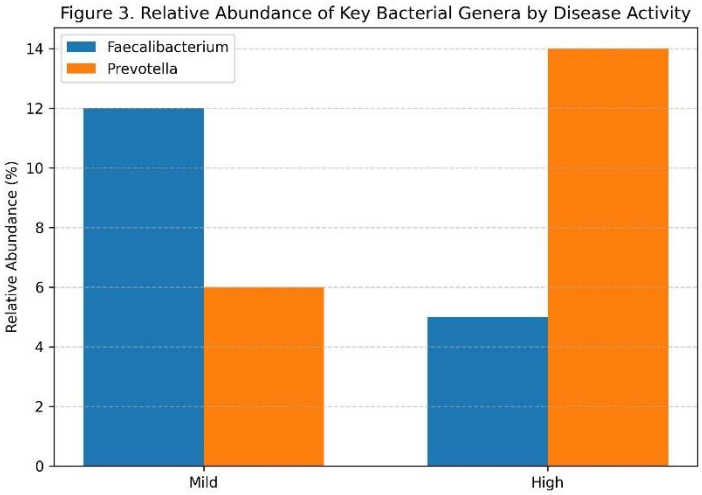


Figure 3. Relative Abundance of Key Bacterial Genera by Disease Activity

Bar chart shows reduced abundance of *Faecalibacterium* and increased *Prevotella* in patients with high disease activity compared to those with mild disease.

Summary of Key Findings

Overall, the results demonstrate a strong association between altered gut microbiota composition and dysregulated immune responses in patients with chronic inflammatory diseases. Distinct microbial patterns were observed between RA and IBD patients, with IBD showing more severe dysbiosis. Reduced microbial diversity and loss of beneficial bacteria were consistently linked to heightened systemic inflammation and immune imbalance.

DISCUSSION

This study provides evidence that alterations in gut microbiota composition are closely linked to immune dysregulation in patients with chronic inflammatory diseases, specifically rheumatoid arthritis (RA) and inflammatory bowel disease (IBD), attending a tertiary care hospital in Khyber Pakhtunkhwa, Pakistan. The findings demonstrate disease-specific microbial signatures, reduced microbial diversity, and strong associations between dysbiosis and systemic inflammatory markers. Together, these results support the concept that gut microbiota plays an integral role in shaping immune responses in chronic inflammatory conditions, even within a distinct South Asian clinical and socio-environmental context.

A key finding of this study was the significant reduction in gut microbial alpha diversity observed in both RA and IBD patients, with a more pronounced loss in the IBD group. Reduced microbial diversity has been widely recognised as a hallmark of chronic inflammatory states and is thought to compromise ecosystem resilience and immune tolerance (Manichanh et al., 2012). The lower Shannon diversity indices observed in IBD patients align with the intestinal localisation of inflammation, where direct mucosal immune activation may exert stronger selective pressures on microbial communities. In contrast, RA patients, despite having systemic inflammation, exhibited relatively preserved diversity, suggesting that gut dysbiosis in extra-intestinal autoimmune disease may follow a different trajectory.

The beta diversity analysis further revealed distinct clustering between RA and IBD patients, indicating that although both conditions are

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

characterised by chronic inflammation, their microbial community structures are not interchangeable. This observation reinforces the notion that disease-specific immune pathways selectively shape microbial ecosystems rather than inflammation alone being the sole determinant (Honda & Littman, 2016). These distinctions are particularly relevant when considering microbiota-targeted interventions, as therapeutic strategies effective in one inflammatory condition may not be universally applicable.

At the taxonomic level, this study identified a relative expansion of *Proteobacteria* in IBD patients, accompanied by a reduction in beneficial butyrate-producing genera such as *Faecalibacterium* and *Roseburia*. The overrepresentation of *Proteobacteria* has been consistently associated with epithelial dysfunction, oxidative stress, and increased inflammatory potential in the gut (Shin et al., 2015). The depletion of SCFA-producing bacteria observed in this cohort is of particular immunological relevance, as SCFAs such as butyrate are critical regulators of regulatory T cell differentiation and intestinal barrier integrity (Arpaia et al., 2013). Their loss may therefore contribute directly to the exaggerated immune activation characteristic of IBD.

In RA patients, the increased abundance of *Prevotella* species observed in this study echoes earlier reports linking *Prevotella copri* to new-onset and active rheumatoid arthritis (Scher et al., 2013). While the mechanistic role of *Prevotella* remains incompletely understood, experimental data suggest that certain strains may promote Th17-mediated immune responses and disrupt mucosal tolerance. The presence of this microbial signature in Pakistani RA patients suggests that this association is not confined to Western populations and may represent a broadly conserved pathogenic pathway.

The immune profiling data provide further support for a functional link between gut microbiota composition and systemic inflammation. Elevated levels of CRP, ESR, TNF-α, and IL-6 were observed in both disease groups, with higher concentrations correlating strongly with reduced microbial diversity and increased abundance of pro-inflammatory taxa. These findings are consistent with the concept that dysbiosis contributes to immune activation through enhanced microbial translocation, pattern recognition receptor signalling, and altered metabolite production (Belkaid & Hand, 2014). The inverse relationship between Shannon diversity index and CRP levels observed in this study underscores the potential of microbial diversity as a biomarker of inflammatory burden.

The observed reduction in anti-inflammatory IL-10 levels, coupled with an increased Th17/Treg cell ratio, provides a plausible immunological mechanism linking dysbiosis to disease activity. Regulatory T cells play a central role in maintaining immune tolerance, and their differentiation is heavily influenced by microbial metabolites (Atarashi et al., 2011). The depletion of commensal bacteria capable of inducing regulatory responses may therefore shift immune balance toward pathogenic inflammation. This imbalance was most evident in patients with high disease activity, suggesting that microbiota-immune interactions may intensify as disease progresses.

Importantly, multivariate analysis demonstrated that gut microbiota diversity remained an independent predictor of inflammatory markers even after adjusting for demographic and clinical variables. This finding strengthens the argument that dysbiosis is not merely a secondary consequence of inflammation or medication use, but may actively contribute to immune dysregulation. In the context of KPK, where delayed diagnosis and prolonged disease duration are common, this relationship may be particularly pronounced due to cumulative environmental and treatment-related pressures on the gut microbiome.

The regional context of this study adds an important dimension to the existing literature. Dietary patterns in Khyber Pakhtunkhwa are characterised by high carbohydrate intake, variable fibre consumption, and frequent use of antibiotics, all of which can influence microbial composition. The microbial signatures observed in this cohort may therefore reflect an interaction

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

between disease pathology and local environmental factors. This underscores the need for region-specific microbiome research rather than extrapolating findings solely from Western populations.

From a clinical perspective, the results of this study have several important implications. First, they support the growing recognition of the gut microbiota as a modifiable factor in chronic inflammatory disease management. While pharmacological therapies remain the cornerstone of treatment, adjunctive strategies aimed at restoring microbial balance—such as dietary modification, targeted probiotics, or prebiotic supplementation—may offer additional benefits. This is particularly relevant in low-resource settings, where access to biologic therapies may be limited.

Second, the strong association between dysbiosis and disease activity suggests that microbiota-based biomarkers could aid in disease monitoring and risk stratification. Non-invasive stool-based assessments of microbial diversity or key taxa may complement existing inflammatory markers and provide insight into treatment response or disease progression. However, longitudinal studies are required to determine whether microbial changes precede clinical flares or simply reflect established inflammation.

Despite its strengths, this study has certain limitations that warrant consideration. The cross-sectional design precludes causal inference, and it remains unclear whether dysbiosis is a driver or consequence of chronic inflammation. Additionally, the absence of a healthy control group limits direct comparison with baseline microbial profiles in the local population. Future studies incorporating healthy controls and longitudinal follow-up would help clarify temporal relationships and disease-specific trajectories.

Another limitation relates to the use of 16S rRNA sequencing, which provides limited resolution at the species and functional levels. Metagenomic and metabolomic approaches could offer deeper insights into microbial functionality and metabolic outputs relevant to immune modulation. Furthermore, while medication use was accounted for in the analysis, residual confounding related to diet and socioeconomic factors cannot be fully excluded.

In conclusion, this study demonstrates that gut microbiota dysbiosis is closely associated with immune imbalance and inflammatory burden in patients with RA and IBD in a tertiary care setting in Khyber Pakhtunkhwa, Pakistan. The findings reinforce the concept of a gut–immune axis that transcends geographical boundaries while highlighting the importance of local context. By contributing region-specific data to the global literature, this research lays the groundwork for future interventional studies aimed at harnessing gut microbiota modulation as a complementary strategy in the management of chronic inflammatory diseases.

CONCLUSION

This study provides compelling evidence that gut microbiota composition is closely linked to immune dysregulation in patients with chronic inflammatory diseases, specifically rheumatoid arthritis and inflammatory bowel disease, in a tertiary care setting in Khyber Pakhtunkhwa, Pakistan. The findings demonstrate that both conditions are characterised by a measurable loss of microbial diversity and distinct disease-specific alterations in bacterial composition, which are strongly associated with systemic inflammation and immune imbalance.

Patients with inflammatory bowel disease exhibited more profound dysbiosis, marked by reduced abundance of beneficial short-chain fatty acid–producing bacteria and expansion of potentially pro-inflammatory taxa. In contrast, rheumatoid arthritis patients showed a different microbial signature, including increased representation of genera previously implicated in autoimmune activation. These disease-specific patterns suggest that gut microbiota alterations are not uniform across chronic inflammatory disorders but instead reflect underlying immunopathological mechanisms unique to each condition.

Importantly, reduced microbial diversity was consistently associated with elevated inflammatory markers and altered cytokine

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

profiles, even after accounting for demographic and clinical variables. The observed imbalance between pro-inflammatory and regulatory immune responses highlights a plausible biological link between intestinal microbial ecology and systemic immune activation. These findings reinforce the concept of a gut–immune axis and suggest that dysbiosis may contribute to disease persistence and severity rather than merely representing a secondary phenomenon.

By focusing on a South Asian population that has been underrepresented in microbiome research, this study adds valuable regional data to the global literature. The results emphasise the need to consider environmental, dietary, and healthcare-related factors when interpreting microbiota–immune interactions and designing therapeutic strategies. Overall, this research supports the growing recognition of gut microbiota as a key factor in the pathogenesis and clinical expression of chronic inflammatory diseases.

RECOMMENDATIONS

Based on the findings of this study, several recommendations can be proposed for clinical practice, research, and public health policy.

First, gut microbiota assessment should be considered as a complementary component in the evaluation of patients with chronic inflammatory diseases. While not intended to replace conventional diagnostic or monitoring tools, microbiota profiling may provide additional insight into disease activity, immune status, and potential treatment response, particularly in patients with refractory or severe disease.

Second, clinicians managing rheumatoid arthritis and inflammatory bowel disease should consider the potential role of gut health in disease management. Dietary counselling aimed at increasing fibre intake and supporting microbial diversity, along with cautious and judicious use of antibiotics, may help preserve beneficial microbial communities. Where appropriate, microbiota-targeted interventions such as probiotics or prebiotics could be explored as adjunctive strategies, although these should be guided by emerging evidence and individual patient characteristics.

Third, future research should prioritise longitudinal and interventional study designs to clarify the causal relationship between gut dysbiosis and immune dysfunction. Long-term follow-up studies could determine whether microbial alterations precede disease flares or predict therapeutic outcomes. Additionally, controlled trials evaluating microbiota-modulating interventions in local populations are needed to establish efficacy and feasibility in resource-limited settings.

Fourth, advanced multi-omics approaches, including metagenomics and metabolomics, should be incorporated into future studies to better characterise microbial functionality and immune-modulatory pathways. Such approaches would provide deeper mechanistic insights and help identify specific microbial metabolites or pathways that could serve as therapeutic targets.

Finally, public health strategies should recognise the broader importance of gut health in chronic disease prevention and management. Improving nutrition, reducing unnecessary antibiotic exposure, and raising awareness about the role of the gut microbiome may contribute to reducing the burden of chronic inflammatory diseases in the region.

In summary, this study highlights the gut microbiota as a significant and potentially modifiable factor in chronic inflammatory diseases. Integrating microbiota-focused perspectives into clinical care and research may open new avenues for improving patient outcomes, particularly in populations where conventional treatment options are limited.

REFERENCES:

Yang W, Cong YZ. Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cellular & Molecular Immunology*. 2021;18:866–877. doi:10.1038/s41423-021-00661-4

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Zhao T, Wei Y, Zhu Y, Xie Z, Hai Q, Li Z et al. Gut microbiota and rheumatoid arthritis: From pathogenesis to novel therapeutic opportunities. *Front Immunol*. 2022;13:1007165. doi:10.3389/fimmu.2022.1007165

Qi P, Chen X, Tian J, Zhong K, Qi Z, Li M et al. The gut homeostasis-immune system axis: Novel insights into rheumatoid arthritis pathogenesis and treatment. *Front Immunol*. 2024;15:1482214. doi:10.3389/fimmu.2024.1482214

Rooney CM, Jeffery IB, Mankia K, Wilcox MH, Emery P. Dynamics of the gut microbiome in individuals at risk of rheumatoid arthritis. *Ann Rheum Dis*. 2024; Early access. doi:10.1136/ard-2024-226362

Ng SC, et al. Targeting gut microbiota dysbiosis in inflammatory bowel disease: Current approaches and future strategies. *Front Med* (Lausanne). 2025;12:1435030. doi:10.3389/fmed.2025.1435030

Iliev ID, Ananthakrishnan AN, Guo CJ. Microbiota in inflammatory bowel disease: Mechanisms of disease and therapeutic opportunities. *Nat Rev Microbiol*. 2025; Published online. doi:10.1038/s41579-025-01163-0

Kaur S, Thakur R. Gut microbiota and inflammatory bowel disease: Mechanisms, clinical implications and future directions. *Mol Biol Rep*. 2026;53:47. doi:10.1007/s11033-025-11195-7

Paudel D, Nair DVT, Joseph G, Castro R, Tiwari AK, Singh V. Gastrointestinal microbiota-directed nutritional and therapeutic interventions for IBD. *Gastroenterol Rep (Oxf)*. 2024;12:goae033. doi:10.1093/gastro/goae033

Zeng MY, Inohara N, Nuñez G. Mechanisms of inflammation-driven dysbiosis in the gut. *Cell Res*. 2017;27(8):881–873. doi:10.1038/cr.2017.83

Chen Z, Zhou L, et al. Dysbiosis of the gut microbiota is associated with autoimmune disorders. *Clin Exp Immunol*. 2023;214(2):241–254. doi:10.1111/cei.14250

Manichanh C, et al. The gut microbiota in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol*. 2012;9(10):599–608. doi:10.1038/nrgastro.2012.157

Scher JU, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *eLife*. 2013;2:e01202. doi:10.7554/eLife.01202

Frank DN, St Amand AL, et al. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A*. 2007;104(34):13780–13785. doi:10.1073/pnas.0706625104

Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157(1):121–141. doi:10.1016/j.cell.2014.03.011

Atarashi K, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*. 2011;331(6015):337–341. doi:10.1126/science.1198469

Arpaia N, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451–455. doi:10.1038/nature12726

Jostins L, et al. Host-microbe interactions shape the genetic architecture of inflammatory bowel disease. *Nature*. 2012;491:119–124. doi:10.1038/nature11582

Neurath MF. Cytokines in inflammatory bowel disease. *Nat Rev Immunol*. 2014;14:329–342. doi:10.1038/nri3661

Garrett WS, Lord GM, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell*. 2007;131(1):33–45. doi:10.1016/j.cell.2007.08.017

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Derrien M, van Hylckama Vlieg JE. Fate, activity, and impact of ingested bacteria within the human gut microbiota. *Trends Microbiol*. 2015;23(6):354–366. doi:10.1016/j.tim.2015.03.002

Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. 2009;9:313–323. doi:10.1038/nri2515

Sartor RB. Mechanisms of disease: Pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol*. 2006;3:390–407. doi:10.1038/ncpgasthep0598

Lan W, Lu Q, Ma W, Jiang Z, Chen Y, Wang Z, et al. Investigating the causal relationship between the gut microbiome and rheumatoid arthritis. *J Transl Med*. 2025;23:187. doi:10.1186/s12967-025-06206-x

Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2:17023. doi:10.1038/sigtrans.2017.23

Butt LAS, Devi J, Deepak P. Strategies for enhancing inflammatory bowel disease care in Pakistan. *Crohn's & Colitis 360*. 2024;6(2):otae027. doi:10.1093/crocol/otae027

Anjum MR. Inflammatory bowel disease in the Pakistani population: An important diagnosis not to be overlooked. *J Pak Soc Intern Med*. 2022;3:195–198.

Husain B, Hussain A, Yaqub Y, Javed F, Usman M, Anjum MA. Study to determine the incidence of rheumatic diseases among rural population of Pakistan. *PJMHSONLINE*. 2021;11:547–550.

Cheng H, Li T, Li C, Su W. Trends and gender disparities in the burden of rheumatoid arthritis in Pakistan. *Sci Rep*. 2025;15:5627. doi:10.1038/s41598-025-05627-4

Ng SC, et al. The Gut Microbiota in Inflammatory Bowel Disease. *Front Cell Infect Microbiol*. 2022;12:733992. doi:10.3389/fcimb.2022.733992

Zhuang X, et al. Effects of prebiotics and probiotics on gut microbiota and immune responses: implications for chronic inflammatory diseases. *Nutrients*. 2024;16:112. doi:10.3390/nu16010112