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Original Article

Comparative analysis of dominant gut microbiota in Inflammatory Bowel Disease patients and healthy individuals: A case-control study

Alireza Ahmadi [a,b], Ebrahim Kouhsari [a,b], Shabnam Razavi [c], Nima Mohamadzadeh [c], Sima Besharat [d], Mohammad Ali Vakili [e], Taghi Amiriani [d,*]

[a] Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[b] Department of Laboratory Sciences, Faculty of Paramedicine, Golestan University of Medical Sciences, Gorgan, Iran
[c] Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[d] Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
[e] Health Management and Social Development Research Center, Department of Biostatistics and Epidemiology, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran

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ABSTRACT

Background: Chronic inflammation in the gut might be linked to microbiota dysbiosis.
Objective: This study aimed to investigate alterations in the gut microbiota composition of adult IBD patients compared to healthy controls.
Methods: This case-control study investigated the relationship between faecal microbiota composition and IBD in adults. Real-time qPCR analysis using bacterial 16S rRNA gene quantified the abundance of six key bacterial groups (Firmicutes, *Lactobacillus* spp., *Bifidobacterium* spp., *Fusobacterium* spp., *Bacteroides fragilis*, and *Faecalibacterium prausnitzii*) in faecal samples from 30 IBD patients (13 Crohn's disease, 17 ulcerative colitis) and 30 healthy controls. A correlation matrix was employed to assess relationships between these bacteria.
Results: Real-time qPCR revealed significant differences (p-value <0.05) in the abundance of several bacterial groups between IBD patients and healthy controls. Firmicutes, Fusobacterium spp., and *B. fragilis* were significantly more abundant (p-value <0.05) in IBD patients compared to controls. Conversely, *Lactobacillus* spp. and *F. prausnitzii* were both significantly less abundant (p-value <0.05) in IBD patients. While some bacterial groups exhibited trends toward higher abundance in either CD or UC patients, these differences were not statistically significant (p-value >0.111). The correlation matrix analysis revealed specific co-occurrence patterns: *Bacteroides* showed a strong negative correlation with *Prevotella*, more abundant in healthy controls, suggesting a shift in dominance in IBD patients. *Lactobacillus spp.* and *F. prausnitzii* exhibited a positive correlation in healthy individuals, indicating their potential cooperative role in maintaining gut homeostasis.
Conclusion: This study identified significant alterations in gut microbiota composition in adult IBD patients compared to healthy controls, with notable differences in the abundance of specific bacterial groups. These findings suggest that gut microbiota dysbiosis may play a critical role in IBD pathogenesis. The identification of specific bacterial imbalances provides a foundation for developing microbiota-based therapies, such as probiotics, prebiotics, and fecal microbiota transplantation, as potential interventions for restoring microbial balance and mitigating disease progression. Further research is needed to translate these insights into targeted therapeutic strategies and to explore their effectiveness in clinical settings.

1. Introduction

The gut microbiota plays a pivotal role in maintaining gastrointestinal health through its contributions to metabolism, immune system regulation, and protection against pathogens [1,2]. Inflammatory Bowel Disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory condition of the gastrointestinal tract that has been increasingly linked to disturbances in the gut microbiota, commonly referred to as dysbiosis [1].

While research has advanced our understanding of the role of gut

* Corresponding author.
E-mail address: dr_amiriani@yahoo.com (T. Amiriani).

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microbiota in IBD, the complexity and variability of microbial changes observed in patients underscore the need for precise and standardized analytical methods [3–5]. Identifying consistent patterns of microbial alterations associated with IBD could help bridge gaps in knowledge and enable the development of microbiota-focused therapies [6,7].

In this study, we utilized real-time quantitative PCR (qPCR) to investigate the differences in gut microbiota composition between adult IBD patients and healthy controls. By focusing on bacterial groups known to influence gut health, this research aims to provide new insights into the role of gut microbial dysbiosis in IBD pathogenesis and its potential implications for therapeutic intervention.

2. Materials and methods

2.1. Study subjects samples collection

This case-control study was conducted at the Golestan University of Medical Sciences in Gorgan, Iran, and included 60 adult participants (30 IBD patients and 30 healthy individuals). Among the IBD patients, 13 had CD and 17 had UC, who visited the Gastroenterology Department of Sayyad Shirazi Hospital between 2022 and 2023. The participants' ages ranged from 18 to 74 years (mean age, 45 ± 35 years), and their body mass indices (BMI) ranged from 18 to 36 kg/m^2.

IBD diagnosis followed the guidelines of the European Crohn's and Colitis Organization. Patients with autoimmune, inflammatory, or infectious diseases, malignancy, or a history of treatment affecting gut microbiota within three months of study participation were excluded. Patients completed a questionnaire providing demographic, clinical, and additional information like birthplace, residence, marital status, occupation, education, ethnicity, personal habits, extraintestinal symptoms, medication intake, and IBD family history.

Informed consent was obtained before specimen and data collection, and 18 matched individuals were included as controls. Stool samples were collected using sterile cups, transferred to a laboratory within 2 h, and stored at −80 °C. The study was approved by the Golestan Ethics Committee (IR.GOUMS.REC.1400.139).

2.2. Genomic DNA extraction

DNA was extracted from the collected samples using a commercial genomic DNA extraction kit, Qiagen QIAamp Mini kit (Qiagen, Crawley, UK). The quality and concentrations of the extracted DNA were then assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To confirm the suitability of the extracted DNA for further analysis, a test PCR was conducted following previously established protocols and utilizing primers universal for bacteria, as described by Hold et al. [8]. The extracted DNA was immediately stored at −20 °C for future analysis.

2.3. Real-time qPCR and microbial quantification

Selected IBD microbiota constituents, including *Firmicutes*, *Lactobacillus* spp., *Bifidobacterium* spp., *Fusobacterium* spp., *Bacteroides fragilis*, and *Faecalibacterium prausnitzii*, were analyzed using specific primers and TaqMan probes targeting the bacterial 16S rDNA gene (as presented in Table 1 [9,10]. The total bacterial concentration was determined as previously described [9,10].

Real-time TaqMan qPCR was performed in duplicate using the RT-qPCR System (Bio-Rad, California, USA) to measure bacterial community differences among the six selected species. The qPCR reaction mixture (20 µl) consisted of 0.5 µl forward/reverse primer, 0.5 µl reverse primer, 0.5 µl TaqMan probe, 12 µl Probe Ex Taq Master Mix (Solis BioDyne, Estonia), 1 µl template DNA, and 5.5 µl sterilized ultra-pure water. qPCR cycling conditions were as follows: initial DNA polymerase activation at 95 °C for 30s, denaturation at 95 °C for 5s, annealing/elongation at 60 °C for 30s, and a final elongation step at 72 °C for 30s (40 cycles each).

2.4. Statistical analysis

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). A parametric two-sample *t*-test and Analysis of Variance (ANOVA) were employed to compare bacterial group abundances between IBD patients and healthy controls. Pearson correlation analysis was conducted to identify potential co-occurrence patterns within the gut microbiota of both groups. *P*-values less than 0.05 were considered statistically significant.

3. Results

The study investigated the composition of faecal microbiota in IBD patients and healthy individuals, focusing on six bacterial groups or strains. Table 2 shown demographic and clinical characteristics of study participants. The differences in composition were analyzed based on gender and BMI, as shown in Figs. 1–4 and Table 3. However, these differences were not found to be statistically significant (*P*-value >0.111). This suggests that the variations observed in the gut microbiota composition between IBD patients and healthy individuals may not be strongly influenced by gender or BMI. Further research considering other factors, such as diet, genetics, or environmental influences,

Table 1
Primers targeting 16SrRNA gene used for determination of microbiota composition by Taq man real-time PCR.

Main target	Primer/Probe	Oligonucleotide sequence (5′–3′)	Size (bp)	Product (bp)
Firmicutes	primer F	CGAACGGGATTAGATACC	18	186
	primer R	CGAATTAAACCACATACTCC	20	
	Probe	CCCCGTCAATTCCTTTGAGTTT	22	
Lactobacillus group	primer F	CCAGGGTATCTAATCCTGTTYG	20	204
	primer R	YCACCGCTACACATGRAGTTCCACT	22	
	Probe	GGTTAACTCGGAGGAAGG	25	
Faecalibacterium prausnitzii	primer F	ATAATGACGGTACTCAACAAGGA	23	171
	primer R	ACAGTTTTGAAAGCAGTTTATGG	23	
	Probe	ACTTCCAACTTGTCTTCCCGCCTG	24	
Bacteroides fragilis	primer F	CGAGGGGCATCAGGAAGAA	19	136
	primer R	CGGAATCATTATGCTATCGGGTA	23	
	Probe	CTTGCTTTCTTTGCTGGCGACCG	23	
Bifidobacterium group	primer F	GGTTAACTCGGAGGAAGG	18	85
	primer R	GTACCGGCCATTGTAGCA	18	
	Probe	CGTCAGATCATCATGCCCCTTACG	24	
Fusobacterium group	primer F	GTATGTCRCAAGCGTTATCC	20	100
	primer R	AACGCAATACRGAGTTGAGC	20	
	Probe	CCTAGACGCGCTTTACGCCCAAT	23	

Abbreviations: primer F (forward primer), primer R (reverse primer), bp (base pair).

Table 2
Characteristics of IBD patients.

Variables	CD (n: 13)	UC (n: 17)
Age, yrs [Median (range)]	50.52 ± 15	49.4 ± 21
Male [N (%)]	9 (69.23)	13 (76.47)
BMI	25.12	23.85
Medication use [N (%)]		
Antibiotics	5 (38.46)	13 (76.47)
Azithromycin	2 (15.3)	4 (23.5)
Cephalosporin	3 (60)	9 (52.9)
Oral 5-ASA	11 (84.6)	15 (88.2)
Immunosuppressant	9 (69.2)	15 (88.2)
Adalimumab	3 (39)	5 (29.4)
PPI	7 (53.8)	16 (94.1)

Abbreviations: CD (Crohn's Disease), UC (Ulcerative Colitis), BMI (Body Mass Index), 5-ASA (5-aminosalicylic acid, PPI (proton pump inhibitors).

may provide more insights into the role of gut microbiota in IBD development and progression.

3.1. Quantitative PCR analysis of Firmicutes, Lactobacillus spp, Bifidobacterium spp, Fusobacterium spp, B. fragilis, and F. prausnitzii

In this case-control study, quantitative polymerase chain reaction (qPCR) analysis was conducted to assess the differences in faecal microbiota composition between IBD patients and healthy individuals, focusing on *Firmicutes*, *Lactobacillus* spp., *Bifidobacterium* spp., *Fusobacterium* spp., *B. fragilis*, and *F. prausnitzii*. After removing outliers, intra-group analysis revealed significant differences in the abundance of two bacterial groups between IBD patients and healthy participants. Figs. 1–4 illustrate the alterations in the composition of faecal microbiota in patients with IBD (CD and UC) and healthy individuals for the six bacterial groups or strains. Findings related to quantification of above bacteria were as follows.

3.2. Firmicutes

As shown in Figs. 1–4, a significant increase in the level of Firmicutes was observed in the IBD groups (CD vs. control and UC vs. control) compared to the healthy group (*P*-value <0.0001). Although the quantity of Firmicutes was higher in the gut microbiota of the UC group than in the healthy subjects, there was no significant difference in Firmicutes frequency between the CD and UC groups (*P*-value = 0.38). These findings suggest that an increased abundance of Firmicutes in the gut microbiota may be associated with the pathogenesis of IBD, particularly in UC cases. The lack of a significant difference in Firmicutes levels between CD and UC patients implies that other bacterial groups or strains might play more critical roles in distinguishing these two forms of IBD.

3.3. Lactobacillus spp

The *Lactobacillus* group was found to be significantly more abundant in healthy individuals compared to IBD subjects (*P*-value <0.0001). Additionally, when comparing *Lactobacillus* frequency between healthy individuals and CD or UC patients separately, the results showed significantly higher levels in the healthy group (*P*-value <0.0001). However, no significant differences in *Lactobacillus* group frequency were observed between CD and UC patients (*P*-value = 0.24). These findings suggest that a higher abundance of *Lactobacillus* in the gut microbiota may be associated with a healthier gut environment and could potentially play a protective role against IBD development. The lack of significant differences in *Lactobacillus* frequency between CD and UC patients indicates that other bacterial groups might be more critical in distinguishing these two forms of IBD.

3.4. Bifidobacterium spp

The *Bifidobacterium* group was significantly more abundant in healthy individuals compared to IBD subjects (*P*-value <0.0001). Furthermore, when comparing *Bifidobacterium* frequency between

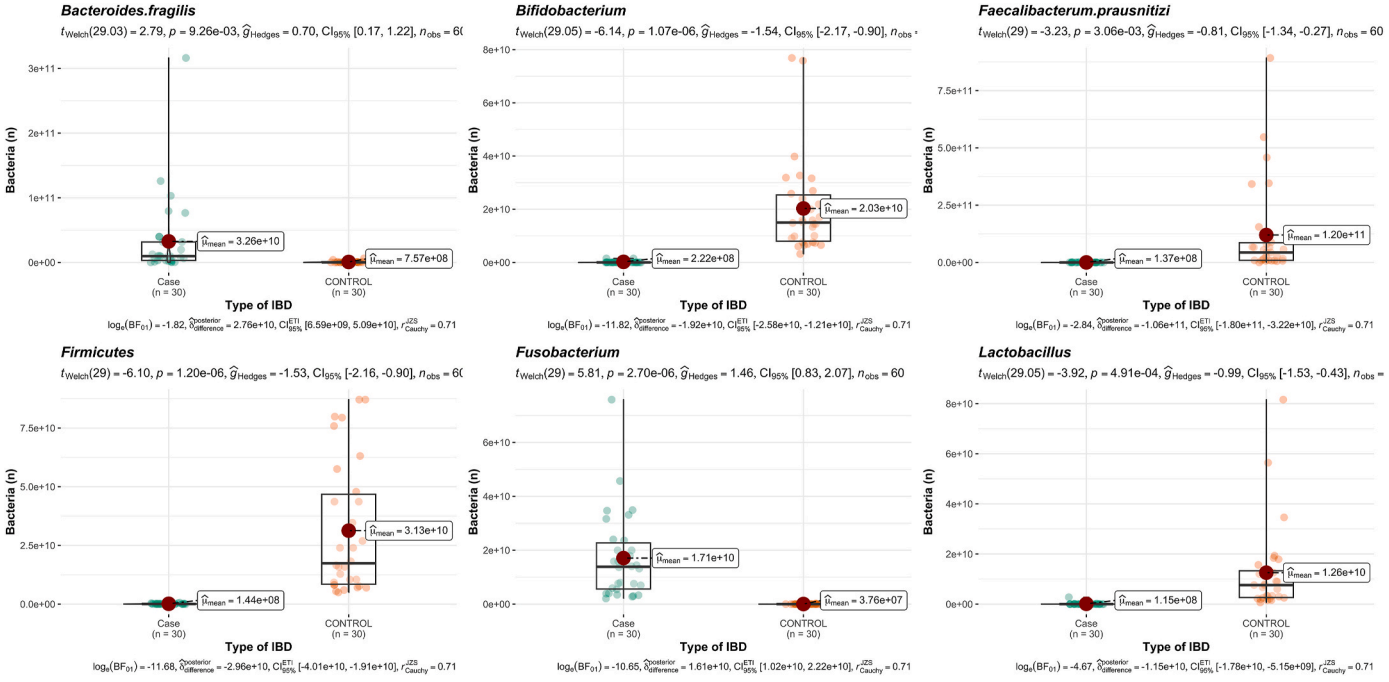

Fig. 1. 16S rDNA qPCR analysis of bacterial composition in patients with IBD and healthy controls. This figure shows the relative abundance of gut bacterial taxa in IBD patients compared to healthy controls. IBD patients exhibit significant shifts in microbial composition, including an increase in pro-inflammatory bacteria and a decrease in anti-inflammatory bacteria. These changes highlight dysbiosis and its potential role in promoting intestinal inflammation and disrupting gut homeostasis. Abbreviation: IBD (Inflammatory Bowel Disease).

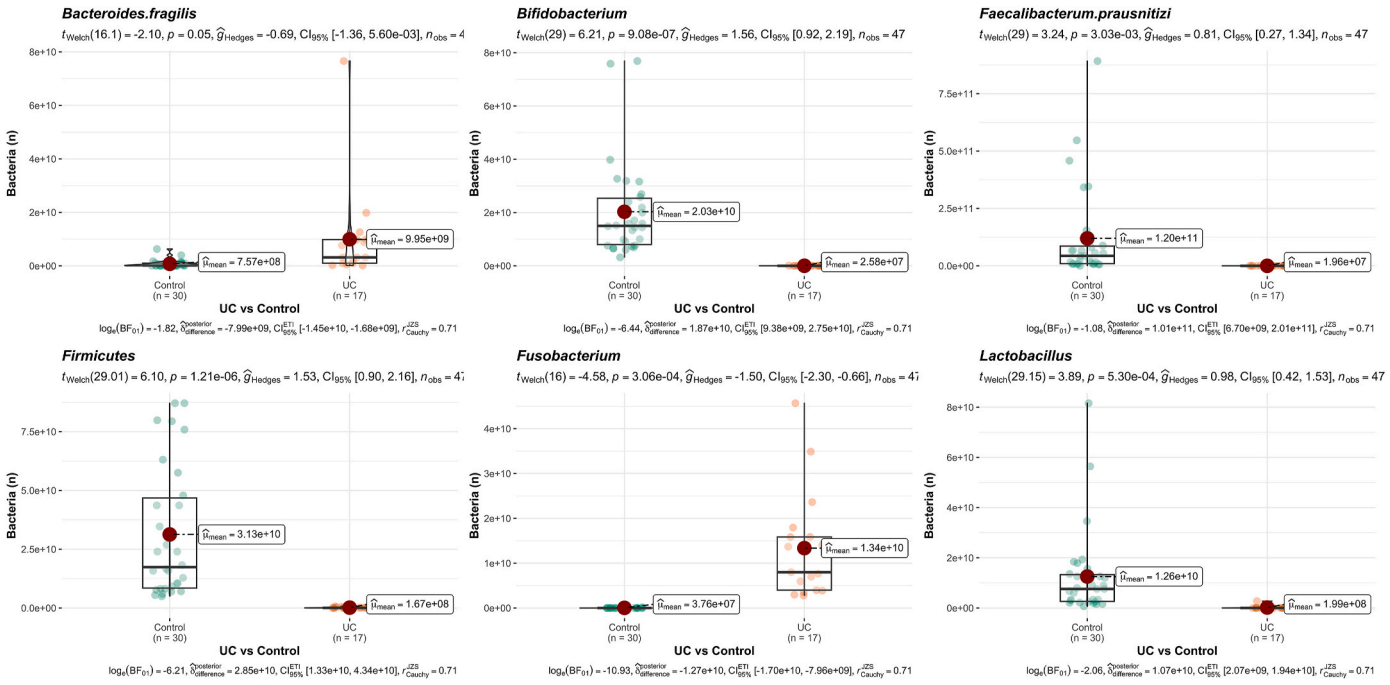

Fig. 2. 16S rDNA qPCR analysis of bacterial composition in patients with UC and healthy controls. This figure compares the gut microbiota composition between UC patients and healthy controls, highlighting significant shifts in bacterial abundance. The analysis reveals microbial imbalances associated with UC, characterized by disruptions in microbial diversity and functions linked to gut health. These findings emphasize the role of dysbiosis in UC pathogenesis and its potential therapeutic implications. Abbreviations: UC (Ulcerative Colitis).

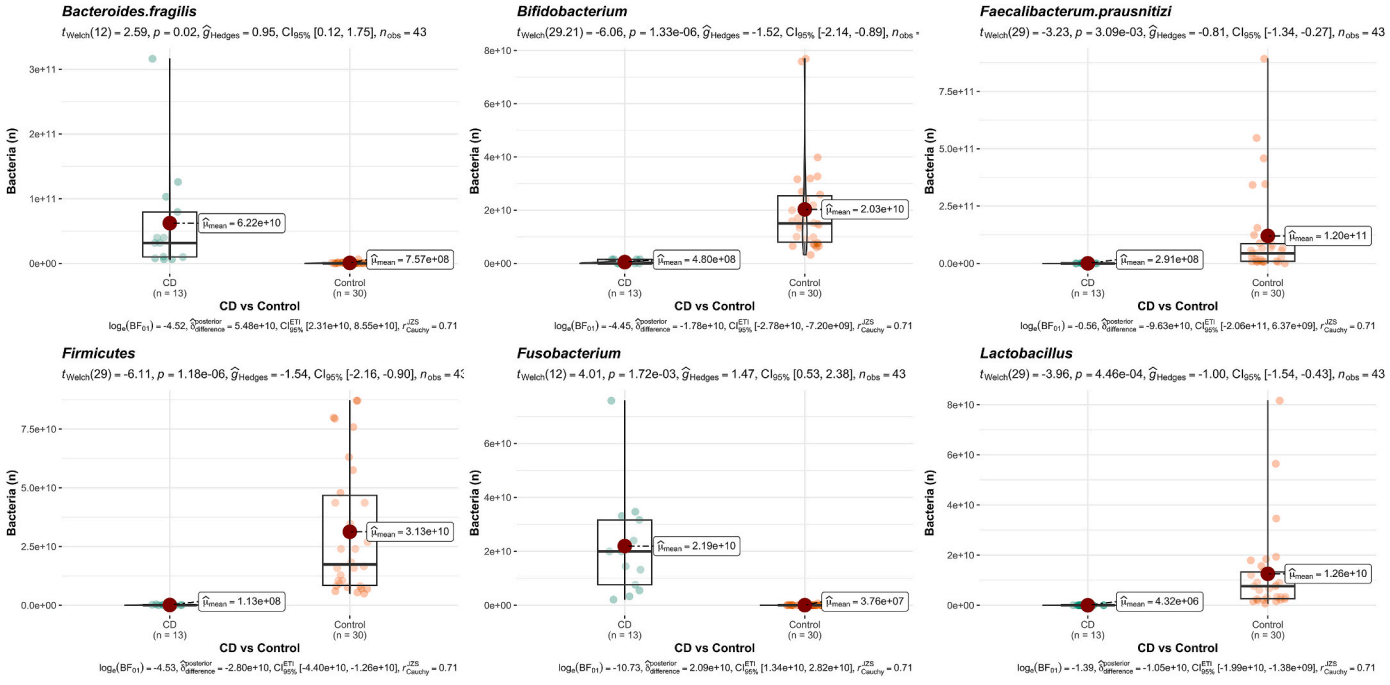

Fig. 3. 16S rDNA qPCR analysis of bacterial composition in patients with CD and healthy controls. This figure illustrates the gut microbiota composition in patients with Crohn's disease (CD) compared to healthy controls. The analysis reveals significant microbial imbalances in CD patients, reflecting disruptions in gut microbial diversity and stability. These findings underscore the role of dysbiosis in CD pathogenesis and its implications for potential therapeutic strategies. Abbreviation: CD (Crohn's Disease).

healthy individuals and CD or UC patients separately, the results showed significantly higher levels in the healthy group (*P*-value <0.0001). Notably, there was a significant difference in *Bifidobacterium* frequency between CD and UC patients (*P*-value = 0.04).

3.5. *Fusobacterium spp*

The abundance of *Fusobacterium* was higher in the gut microbiota of IBD patients (CD vs. control and UC vs. control) compared to healthy subjects, with a significant difference in *Fusobacterium* frequency

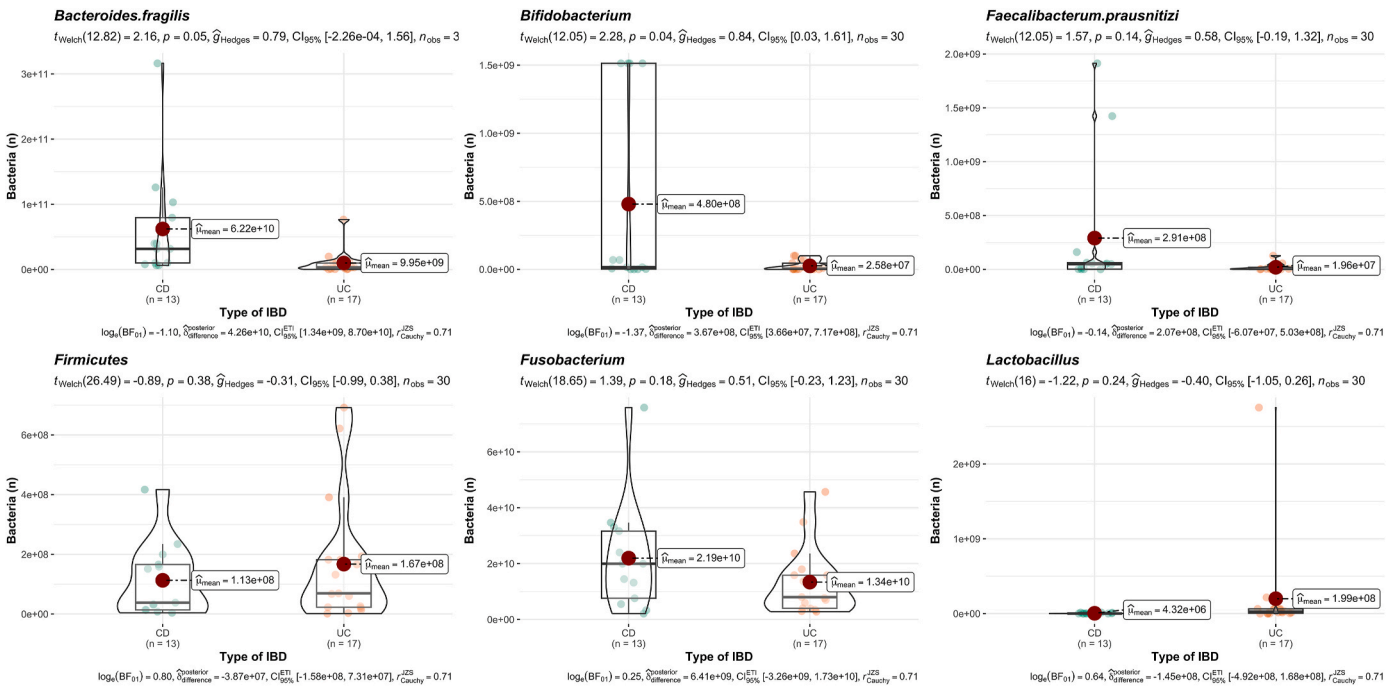

Fig. 4. 16S rDNA qPCR analysis of bacterial composition in patients with UC and CD. This figure compares the gut microbiota composition between patients with ulcerative colitis (UC) and Crohn's disease (CD) using 16S rDNA qPCR analysis. The results reveal distinct microbial profiles for UC and CD, highlighting differences in bacterial abundance and diversity that may reflect disease-specific dysbiosis. These findings provide insights into the unique microbial disruptions associated with each condition and their implications for targeted therapeutic approaches. Abbreviations: CD (Crohn's Disease), UC (Ulcerative Colitis).

Table 3
Quantification of bacterial faecal microbiota in IBD patients and healthy individuals.

Bacterial species	IBD (Mean ± SD)	CD (Mean ± SD)	UC (Mean ± SD)	Control (Mean ± SD)
Firmicutes	10.22 ± 10.46	8.20 ± 8.30	10.47 ± 10.52	10.17 ± 10.32
***Lactobacillus* group**	8.06 ± 8.70	6.64 ± 6.73	8.30 ± 8.82	10.10 ± 10.24
F. prausnitzii	8.14 ± 8.63	8.46 ± 8.79	7.29 ± 7.51	11.08 ± 11.31
B. fragilis	10.51 ± 10.80	10.79 ± 10.93	10.00 ± 10.26	8.88 ± 9.13
***Bifidobacterium* group**	8.35 ± 8.71	8.68 ± 8.86	7.41 ± 7.56	10.31 ± 10.25
***Fusobacterium* group**	10.23 ± 10.21	10.34 ± 10.29	10.13 ± 10.08	7.58 ± 8.12

Abbreviations: CD (Crohn's Disease), UC (Ulcerative Colitis), IBD (Inflammatory Bowel Disease).

between the two study groups (*P*-value <0.0001). While the quantity of *Fusobacterium* was higher in the UC group than in the CD group, there was no significant difference in *Fusobacterium* frequency between CD and UC patients (*P*-value = 0.18).

3.6. *B. fragilis*

The abundance of *B. fragilis* was higher in the gut microbiota of IBD patients (CD vs. control and UC vs. control) compared to healthy subjects, with a significant difference in *B. fragilis* frequency between the two study groups (*P*-value <0.05). Although the quantity of B. fragilis was higher in the UC group than in the CD group, there was no significant difference in *B. fragilis* frequency between CD and UC patients (*P*-value = 0.05).

3.7. *F. prausnitzii*

F. prausnitzii was found to be significantly more abundant in healthy individuals compared to IBD patients (*P*-value <0.0001). Further analysis showed that *F. prausnitzii* levels were significantly higher in healthy individuals when compared to CD or UC patients individually (*P*-value <0.0001). However, there was no significant difference in *F. prausnitzii* frequency between CD and UC patients (*P*-value = 0.14).

3.8. *The correlations between concentration of the bacterial species and BMI*

This study employed a correlation matrix to assess the relationships between the abundance of six key gut bacterial groups (Firmicutes, *Lactobacillus* spp., etc.) within the fecal microbiota of the study participants (Fig. 5). The correlation coefficient (r), ranging from −1 to 1, is displayed within each square of the matrix, indicating the strength and direction of the association between two bacterial groups. Positive values (closer to 1) suggest that the abundance of these bacteria tends to increase or decrease together, while negative values (closer to −1) imply an opposing trend. The *p*-value (p) accompanying each correlation coefficient signifies the statistical significance of the observed relationship. A low *p*-value (typically less than 0.05) indicates that the correlation is unlikely to be due to chance. For instance, a correlation coefficient of 0.44 (p = 0.01) between Firmicutes and *Bifidobacterium* spp. suggests a moderate positive association, meaning their abundance might exhibit a tendency to rise or fall together. The low *p*-value (0.01) implies this association is statistically significant. Analysis of the entire correlation matrix allows researchers to explore potential co-occurrence patterns or dependencies between these various gut bacterial groups within the human microbiome.

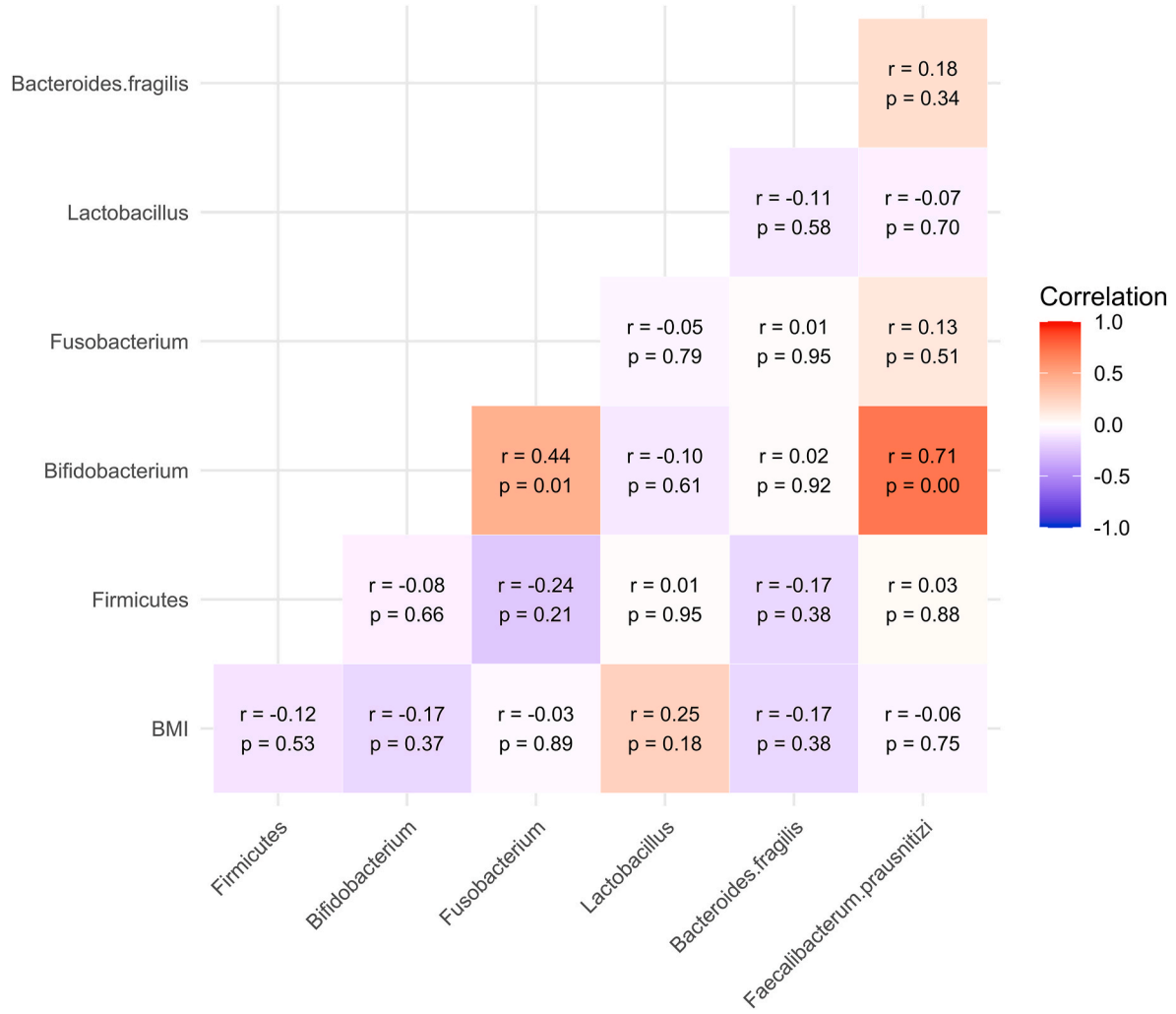

Fig. 5. The correlations between concentration of the bacterial species and BMI. This figure demonstrates the relationships between the concentrations of various bacterial species and body mass index (BMI). The analysis reveals specific bacterial taxa exhibiting significant positive or negative correlations with BMI. These findings suggest that alterations in gut microbiota composition may be associated with BMI variations, providing potential insights into microbiota-related mechanisms influencing metabolic health.

4. Discussion

The results of this study contribute to the growing body of evidence supporting the pivotal role of gut microbiota dysbiosis in the pathogenesis of IBD, which includes both CD and UC. Through real-time qPCR analysis, we have identified significant alterations in the abundance of various bacterial groups in adult IBD patients when compared to healthy controls.

In comparison with other studies, our findings both corroborate and expand upon the existing understanding of gut microbiota dysbiosis in IBD patients. Several studies have reported a decreased abundance of beneficial bacteria, such as *Lactobacillus* spp. and *F. prausnitzii*, in IBD patients, which is consistent with our observations. For instance, a study by Machiels et al. (2017) [11] found a lower abundance of *Lactobacillus* spp. in IBD patients, while *F. prausnitzii* depletion has been reported in various studies, including those by Willing et al. (2009) [12] and Sokol et al. (2009)([13]). These findings underscore the potential importance of these bacterial groups in maintaining intestinal homeostasis and mitigating inflammation.

We also observed an increased abundance of Firmicutes, *Fusobacterium* spp., and *B. fragilis* in IBD patients, which is in line with previous research. Manichanh et al. (2006) [14] reported an increase in Firmicutes in IBD patients, while Geirnaert et al. (2014) [15] found a higher abundance of *Fusobacterium* spp. in IBD patients, particularly in CD patients. *B. fragilis*, known for its pro-inflammatory properties, has been previously linked to IBD pathogenesis in studies by Rizzetto et al. (2018) [16] and Wang et al. (2021) [17]. These findings collectively suggest that an increased abundance of these bacterial groups may play a role in promoting intestinal inflammation in IBD.

Unlike some previous studies, we did not observe significant differences in microbiota composition between CD and UC patients. This finding is in line with a study by Joossens et al. (2011)([18]), which also found no significant differences in overall microbial diversity between CD and UC patients. However, Morgan et al. (2012)([19]) has reported unique microbial signatures for each IBD subtype, suggesting that larger sample sizes may be needed to uncover potential differences in gut microbiota composition.

Our study contributes to the understanding of the underlying mechanisms involved in gut microbiota dysbiosis in IBD([20]). The decreased abundance of beneficial bacteria like *Lactobacillus* spp. and *F. prausnitzii* may disrupt the gut barrier function and promote inflammation through the production of pro-inflammatory cytokines [21]. These bacteria are known for their anti-inflammatory and protective properties, and their depletion may lead to a disruption of gut homeostasis and an exacerbation of intestinal inflammation. This is further supported by the inverse correlation observed between *Lactobacillus* spp.

and *F. prausnitzii* levels and the severity of IBD symptoms [22–24].

On the other hand, the increased abundance of Firmicutes, *Fusobacterium* spp., and *B. fragilis* may contribute to IBD pathogenesis by inducing the production of inflammatory mediators and impairing the host immune response.

Our correlation matrix analysis has uncovered potential co-occurrence patterns among various bacterial groups in both IBD patients and healthy controls. This finding emphasizes the complexity of microbial interactions within the gut ecosystem and suggests that the pathogenesis of IBD may involve intricate networks of microbial relationships. Future studies should delve deeper into these interactions and their functional implications to unravel the mechanisms underlying the development and progression of IBD.

The lack of significant microbiota differences between Crohn's disease (CD) and ulcerative colitis (UC) may be explained by several factors. Both conditions share overlapping pathophysiological mechanisms, such as immune dysregulation and inflammatory responses, which can result in similar microbial alterations. Heterogeneity within CD and UC subtypes, such as inflammation location, may obscure differences. Additionally, shared treatments like immunosuppressants or corticosteroids may influence microbiota composition, masking disease-specific variations. Technical limitations, such as the resolution of 16S rDNA qPCR, may also reduce the ability to detect subtle differences. Functional overlap between the microbial communities in CD and UC may lead to similar disruptions in metabolic or immune interactions, even if taxonomic differences exist. Finally, shared environmental or lifestyle factors, including diet, may homogenize microbiota profiles across IBD patients.

This study reveals gut microbiota imbalances in IBD, highlighting opportunities for microbiota-based therapies. Restoring microbial balance through probiotics, dietary changes, or fecal microbiota transplantation could complement existing treatments. Additionally, these findings may aid in developing biomarkers for personalized therapeutic approaches, emphasizing the importance of targeting gut microbiota in managing IBD.

This study has several limitations that should be considered when interpreting the findings. The relatively small sample size may limit the generalizability of the results, and the geographic specificity of the cohort may not fully capture global variations in gut microbiota composition. Additionally, the use of 16S rDNA qPCR provides taxonomic insights but lacks resolution for functional analyses of microbial communities. Future studies with larger, more diverse cohorts and multi-omics approaches are needed to validate and expand these findings.

To build on these findings, future research should include longitudinal studies to track dynamic changes in gut microbiota over the course of IBD progression and treatment. Mechanistic experiments, such as in vitro and in vivo models, are essential to understand the causal relationships between specific microbial taxa and disease pathogenesis. Additionally, integrating multi-omics approaches, including metagenomics and metabolomics, could provide deeper insights into the functional roles of microbial communities. These steps will help translate microbiota-based research into effective therapeutic strategies for IBD.

5. Conclusions

Our study provides further evidence on the pivotal role of gut microbiota dysbiosis in IBD pathogenesis, revealing significant alterations in bacterial composition in adult IBD patients compared to healthy controls. These findings underscore the potential importance of gut microbiota in IBD management and support the need for further research on the role of dysbiosis in disease development and progression. Ultimately, a deeper understanding of gut microbiota and its interactions with the host may pave the way for the development of innovative therapeutic strategies aimed at restoring gut homeostasis and improving the management of IBD. Furthermore, our findings suggest potential avenues for future research, including the exploration of subtype-specific variations in gut microbiota composition, the functional roles of various bacterial groups, and the development of microbiota-based interventions, such as probiotics, prebiotics, fecal microbiota transplantation, and dietary modifications.

CRediT authorship contribution statement

Alireza Ahmadi: Writing – original draft, Data curation, Conceptualization. **Ebrahim Kouhsari:** Writing – original draft, Supervision, Methodology, Investigation. **Shabnam Razavi:** Conceptualization. **Nima Mohamadzadeh:** Validation, Methodology, Investigation. **Sima Besharat:** Conceptualization. **Mohammad Ali Vakili:** Formal analysis. **Taghi Amiriani:** Writing – review & editing, Methodology, Investigation, Conceptualization.

Consent to participate declaration

Not applicable.

Ethical approval

This project was approved by the Golestan University Human Ethics committee (Ethical code: IR.GOUMS.REC.1400.139).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

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