

Original Article

Longitudinal Analyses of Gut-Associated Bacterial Microbiota in Ulcerative Colitis Patients

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Background: The normal colonic microbiota is associated with the etiology of ulcerative colitis (UC). Several bacterial species are associated with the initiation and amplification of disease process. However, the etiology and mechanism of UC are poorly understood. The present study aimed to investigate, characterize, and compare the main composition of the mucosa-associated intestinal microflora in colonoscopic biopsy specimens of UC and non-UC patients.

Methods: Aerobic and facultative-anaerobic mucosa-associated bacteria were isolated and diagnosed from colonoscopic biopsy specimens of 40 UC patients and 40 patients without UC. Patients were selected as control from the same centers and colonoscopy was carried out for other reasons (mainly colorectal screening). Isolation and characterization for aerobic and facultative-anaerobic intestinal bacteria were carried out by conventional culture techniques. DNA extraction from biopsies and polymerase chain reaction (PCR) amplification of bacterial 16S rRNA with gene-targeted and species-specific primers was performed for detection of anaerobic bacterial species.

Results: Several species of mucosa-associated aerobic and facultative anaerobic bacteria were found in biopsy specimens and there were no significant differences between UC patients and non-UC patients. Our investigation for detection of the anaerobic intestinal flora showed *Faecalibacterium prausnitzii*, *Prevotella*, and *Peptostreptococcus productus* were the predominant microflora in controls and have significant differences ($P = 0.002, 0.025$ and 0.039 , respectively).

Conclusion: This is the first investigation of the intestinal mucosa-associated microflora in patients with UC in Iran. These results, although limited by sample size, allow a better understanding of changes in mucosa-associated bacterial flora in these patients, showing that decrease of *Faecalibacterium prausnitzii*, *Prevotella*, and *Peptostreptococcus productus* in the intestinal tract may translate into a reduction in the important role of this beneficial bacterial species, which can lead to reduced protection of the gut mucosa and UC development.

Keywords: Inflammatory bowel disease, Polymerase chain reaction, Ribosomal RNA, Ulcerative colitis

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Introduction

The gut microbiota has an essential and important role in the protection of mucosa,¹ and the gut contains trillions of microbes that influence human health.² Inflammatory bowel diseases (IBDs) are chronic gastrointestinal diseases, which include 2 main clinical phenotypes, Crohn's disease (CD) and ulcerative colitis (UC) and the latter seems to be more common in Iran.³ UC is characterized by mucosal inflammation, which is limited to the colon, and it begins in the rectum and extends to adjacent parts. In contrast, CD occurs at any location in the gastrointestinal tract, from the mouth to the anus, and it is more prevalent in young adults.⁴ Therefore, patients would be dealing with their illness for a long time. UC affects the large bowel where bacteria are more than any other part of the gut and the flow rate of luminal contents is slowest.⁵ UC develops in about 2 per 10 000 adults yearly.^{4,6} Although UC has been known as

a medical entity since 1859,⁷ the etiology is still unclear.⁸ However, it is commonly accepted that the cause may lay in host genetics,⁹ environmental factors,¹⁰ and unregulated immune responses.¹¹

Previous studies suggested an association between gut microbiota and UC progress,¹² as well as the association between bacteria belonging to the common colonic microbiota and the etiology and process of UC.⁹ Bacteria growing on the gut wall may lead to UC by colonizing pathogenic organisms on the epithelial surface and attacking the mucosa, or alternatively by inhibiting the adhesion sites of non-pathogenic commensal species on the mucosa and avoiding the attachment of bacteria.¹³

However, until now, no single microbial agent has been found in relation to the development of UC.¹⁴ UC is associated with a breakdown in the balance between the different protective and harmful intestinal bacteria

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and a decrease in bacterial diversity.¹⁵ The beneficial bacterial strains, such as *Bifidobacterium* and *Lactobacilli*, were generally absent from mucosa-associated bacterial flora in patients with active UC.¹⁶ On the other hand, an increased mucosal concentration of Gram-negative anaerobic bacteria, especially *Escherichia coli*, *Fusobacterium varium*, and *Bacteroides* spp., along with a high frequency of *Peptostreptococcus* spp. has recently been shown.¹⁷ In addition, genetic predispositions and inflammation of the host induce a change in composition and metabolic process of microbial populations, which has recently been termed as dysbiosis.¹⁸ Conversely, the field remains descriptive, and some of the most basic questions about the role of the gut microbiota in IBD remain unanswered. New technology explains the function of bacterial species, which might be considered intestinal dysbiosis, as an initiation and activation factor of UC.⁴

Many studies have observed imbalances or dysbioses in the GI microbiomes of UC patients.¹⁹ Therefore, the present research aimed to investigate and compare differences between the mucosa-associated microbiota in colonoscopy biopsy specimens from patients with and without UC (depending on detection of 16s rRNA-based amplification analysis).

Materials and Methods

Sample Size

According to a literature review, we found that in the UC group, 50% of patients are positive for *Fusobacterium prausnitzii* but in non-UC patients (control), this bacteria is present 85% of the time. For sample size estimation, the following formula was used:

$$n = \frac{\left(Z_{1-\frac{\alpha}{2}} \sqrt{2\bar{P}(1-\bar{P})} + Z_{1-\beta} \sqrt{P_0(1-P_0) + P_1(1-P_1)} \right)^2}{(P_1 - P_0)^2}$$

For a 95% confidence level and power of 80%, we needed at least 36 people in each group.

Treatment of Biopsy Specimens

In the present study, 40 consecutive patients with a known diagnosis of UC were selected from Masoud clinic and Shariati hospital of Tehran University of Medical Sciences, Iran. Forty patients were also selected as the control group from the same centers in which colonoscopy was carried out for other reasons (mainly colorectal screening). Subjects were gathered from December 2015 to December 2016.

Patients who had received antibiotics within 3 months or who had received corticosteroids within 4 weeks before the study were excluded. In addition, subjects who did not consent to the study were excluded. Endoscopic biopsies were taken from the involved mucosa of UC patients and from the rectum of non-UC patients. Each biopsy was placed in 1 mL of sterile phosphate buffer saline solution.²⁰ For the bacteriological study, specimens were immediately processed in the Microbiology Laboratory in Faculty of Veterinary Medicine at University of Tehran, Iran. Biopsy washing

was done according to what mentioned in.²¹ Briefly, biopsy samples (15 mg of each sample) were first washed with 500 μ L of physiological saline with 0.016% dithioerythritol to remove the mucus and then washed three times with 500 μ L of physiological saline by shaking for 30 s each time. Then, the biopsy specimens were hypotonically lysed by overtaxing for 30 min in 500 μ L distilled water to analyze mucosal aerobic and facultative-anaerobic bacteria or were processed for DNA extraction to evaluate the molecular detection of anaerobic bacteria.

Culture Conditions for Aerobic and Facultative Anaerobic Bacteria

In order to study the aerobic and facultative anaerobic bacteria, the cell debris after hypotonic lysis (100 μ L) was cultured in blood agar and MacConkey agar at 37°C overnight. A single colony was chosen for further investigations, examination, and identification. Biochemical characterization was performed using TSI, urea agar, Simon citrate agar, indole medium, glucose, methyl red, motility test, and oxidase test.

DNA Extraction

After the fourth washing, the biopsy specimens were incubated with 180 μ L (ATL) buffer and 20 μ L proteinase K was added. Finally, specimens were incubated at 55°C for 2 hours and then 20 μ L lysozyme was added for a further 2 hours at 37°C. DNA was extracted with the DNeasy Tissue Kit (MBST, Iran) based on the manufacturer's instructions. The quality and quantity of the extracted DNA were determined by agarose gel electrophoresis and confirmed by measuring the absorbance at 260 nm using a NanoDrop spectrophotometer ND-1000 (Thermo Fisher Scientific, Wilmington, DE, USA).

Polymerase Chain Reaction (PCR) Primers and Condition

The species-specific primers (Takapouzi, Iran) for anaerobic bacteria detection were listed in Table 1. The reactions mixture of PCR were 25 μ L in total volume containing 6.5 μ L of distilled water (dH₂O), 2.5 μ L 10X buffer (200 mM Tris HCl [pH 8.4], 500 mM KCl) supplied with 1 ml of 50 mM MgCl₂, 1 μ L of each primers (forward 0.5 μ L and reverse 0.5 μ L from 10 pmol concentration), 4 μ L of genomic DNA, 1 μ L dNTP from 10 μ M concentration, 1 μ L MgCl₂ 50 μ M, and 0.5 μ L Taq DNA Polymerase (2.5 unit). The PCR was performed using a DNA thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The thermocycler was programmed for each reaction as mention in Table 2. The PCR products were electrophoresed in 1.5% agarose (Fermentas) for 1 hours at 100 V and the gels were stained with ethidium bromide (2 mg/mL) for 15 minutes. Then they were photographed under UV transilluminator (BIORADE, UK) for visualized fluorescent bands.

DNA Sequencing

As no control strains were available for the investigated genes, the polymerase chain reaction (PCR) products were

Table 1. Primers Used in This Study

Bacteria	Primer	Sequence (5'-3')	PCR Product(bp)	Reference
<i>Prevotella</i>	g-Prevo-F	5'- CACRGTAAACGATGGATGCC -3'	527-529	22
	g-Prevo-F	5'- GGTCGGGTTGCAGACC -3'		
<i>Bifidobacterim</i> spp.	g-Bifid-F	5'- CTCCTGAAACGGGTGG-3'	549-563	22
	g-Bifid-R	5'- GGTGTTCTTCCCGATATCTACA-3'		
<i>Clostridium butyrim</i>	-	5'- GTGCCGCCGTAACGCATTAAGTAT-3'	213	23
	-	5'- ACCATGCACCACCTGTCTTCTGCC-3'		
<i>Lactobacilli</i>	-	5'- GATAGAGGTAGTAACCTGGCCTTAc-3'	390	17
	-	5'- GCGGAAACCTCCCAACA-3'		
<i>Bacteroides fragilis</i> group	g-Bfra-F	5'- ATAGCCTTTCGAAAGRAAGAT-3'	501	22
	g-Bfra-R	5'- CCAGTATCAACTGCAATTTTA-3'		
<i>Bacteroides thetaiotaomicron</i>	BT-1	5'- GGCAGCATTTTCAGTTGCTTG-3'	423	24
	BT-2	5'- GGTACATACAAAATCCACACGT-3'		
<i>Bacteroides vulgatus</i>	BV-1	5'- GCATCATGAGTCCGCATGTTCC-3'	287	24
	BV-2	5'- TCCATACCCGACTTTATTCCTT-3'		
<i>Bacteroides distasonis</i>	BD-1	5'- GTCGGACTAATACCGCATGAA -3'	273	24
	BD-2	5'- TTACGATCCATAGAACCTTCAT-3'		
<i>Bifidobacterium adolescentis</i>	BIA-1	5'- GGAAAGATTCTATCGGTATGG-3'	244	24
	BIA-2	5'- CTCCCAGTCAAAAGCGGT-3'		
<i>Bifidobacterium longum</i>	BIL-1	5'- GTTCCCGACGGTCGTAGAG-3'	153	24
	BIL-2	5'- GTGAGTTCCTCCGCATAATCC -3'		
<i>Eubacterium biforme</i>	EBI-1	5'- GCTAAGGCCATGAACATGGA -3'	46	24
	EBI-2	5'- GCCGTCCTCTTCTGTTCTC-3'		
<i>Fusobacterium prausnitzii</i>	FPR-1	5'- AGATGGCCTCGCGTCCGA -3'	199	24
	FPR-2	5'- CCGAAGACCTTCTTCTCC-3'		
<i>Peptostreptococcus products</i>	PSP-1	5'- AACTCCGGTGGTATCAGATG -3'	268	24
	PSP-2	5'- GGGGCTTCTGAGTCAGGTA-3'		

Table 2. PCR Amplification Programs for This Study

Bacteria	Initial Denaturation	Cycling (35 Cycles)			Final Extension
		Denaturation	Annealing	Extension	
<i>Provetella</i> spp.	95°C for 7 min	95°C for 1 min	55°C for 1 min	72°C for 1 min	72°C for 7min
<i>Bifidobacteriumn</i> spp.	95°C for 7 min	94°C for 30 s	57°C for 30 s	72°C for 45 s	72°C for 5 min
<i>Clostridium butyricum</i>	95°C for 2 min	95°C for 1 min	67°C for 1 min	72°C for 45 s	72°C for 5 min
<i>Lactobacilli</i> spp.	95°C for 2 min	95°C for 1 min	63°C for 1 min	72°C for 45 s	72°C for 5 min
<i>Bacteroides fragilis</i> group	95°C for 7 min	94°C for 20 s	57°C for 20 s	72°C for 30 s	72°C for 5min
<i>Bacteroides thetaiotaomicron</i>	95°C for 7 min	94°C for 20 s	60°C for 20 s	72°C for 30 s	72°C for 5 min
<i>Bacacteriobes vulgatus</i>	95°C for 7 min	94°C for 30 s	55°C for 10 s	72°C for 35 s	72°C for 2 min
<i>Bacteriods distasonis</i>	95°C for 7 min	94°C for 20 s	55°C for 10 s	72°C for 35 s	72°C for 2 min
<i>Bifidobacter odelescentis</i>	95°C for 7 min	94°C for 30 s	55°C for 30 s	72°C for 45 s	72°C for 5 min
<i>Bifidobacterium longum</i>	95°C for 7 min	94°C for 30 s	57°C for 30 s	72°C for 45 s	72°C for 5 min
<i>Eubacterium biforme</i>	95°C for 7 min	94°C for 20 s	56°C for 10 s	72°C for 3 5 s	72°C for 2 min
<i>Fusobacterium prausnitzii</i>	95°C for 7 min	95°C for 1 min	62°C for 1 min	72°C for 1 min	72°C for 7 min
<i>Peptostreptococcus produs</i>	95°C for 7 min	95°C for 1 min	58°C for 1 min	72°C for 1 min	72°C for 7 min

confirmed by DNA sequencing.

Statistical Analysis

For comparisons between UC and non-UC groups, the result of bacterial detection was considered as positive or negative for each individual. Chi-square, Fisher exact test and the *t* test were used for univariate analysis. Odds ratio (OR), confidence interval (95% CI) and logistic regression were used to determine the association between bacterial species and UC in patients elder and younger than 35 years of age. In addition to age, the sex was also taken in to consideration. *P* value <0.05 was considered statistically

significant. Statistical analysis of the data was conducted using the SPSS 16.0 software program.

Results

Age and Gender Susceptibility

There was no strong evidence to relate disease duration in UC and non-UC groups with different ages and genders. The median (range) age and gender were 41.55 and 47.20 years for UC and non-UC groups, while the male/female ratio was 18/22 for UC patients and 14/26 for Non-UC patients (Table 3).

Table 3. Characteristics of UC and Non-UC Patients

Characteristic	Value for Group	
	UC Patients	Non-UC Controls
No. of subjects	40	40
Gender (male/female)	18/22	14/26
Age range (y) (mean ± SEM)	(41.55 ± 2.754)	(47.20 ± 2.367)

Abbreviations: UC, ulcerative colitis.

Identification of Aerobic and Facultative-anaerobic Bacterial From Biopsy Colon

Among bacteria isolated from biopsy specimens of all groups (UC and non-UC patients), 97.5% (78 out of 80) were positive for aerobic and facultative-anaerobic bacterial culture. When the occurrence of bacterial species in each patient group was analyzed, Gram-negative organisms in samples of UC patients were identified as *E. coli* 12(30.0%), *Klebsiella* spp. 9 (22.5%), and *Proteus* spp. 5 (12.5%), while in non-UC patients *E. coli*, *Klebsiella* spp. and *Proteus* spp. were 10 (25%) 9 (22.5%), 12 (30%) and 10 (25%), respectively. On the other hand, Gram-positive bacterial populations in UC patients were *Staphylococcus* spp. 5 (12.5%), *Streptococcus* spp. 4 (10%), and *Enterococcus* spp. 2(5%), but in the non-UC patients were *Staphylococcus* spp. 5 (12.5%), *Streptococcus* spp. 3 (7.5%), and *Enterococcus* spp. 2 (5%). Our results showed non-significant differences between aerobic and facultative anaerobic bacterial population between UC and non-UC patients (Table 4).

After adjusting OR for age and sex, our results revealed no significant difference between aerobic bacterial species and UC (Table 5).

Percentage of Patients in Anaerobic Bacterial Groups' PCR Assay

Variety of mucosa-associated anaerobic flora from biopsy specimens were assessed by PCR. Thirteen primer sets for different mucosal bacteria were used to analyze biopsy specimens from the UC and non-UC patients (Table 1). The selection of the anaerobic bacterial groups was based

on previous studies that treated the relative frequency of bacterial species found in the human intestinal tract associated with the mucosa of healthy people and patients with IBD.^{17,22-24}

All primer sets were highly specific and gave positive results only for the corresponding target bacteria with the expected product size. From these positive results, 3 bacterial spp had significant differences; *Faecalibacterium prausnitzii*, *Prevotella* and *Peptostreptococcus productus*, (*P* values 0.002, 0.025 and 0.039 respectively, Table 6). But, after adjusting OR for age and sex the *Prevotella* did not show any significant difference (Table 7). While, 10 bacterial population groups had non-significant differences (*Bifidobacterium* spp, *B. adolescentis*, *B. longum*, *Bacteroides fragilis* group, *B. thetaiotaomicron*, *B. distasonis*, *B. vulgatus*, *E. bifforme*, *Lactobacilli* and *Clostridium butyricum*, Table 6). Furthermore, the OR of the 10 bacterial population groups mentioned above indicate non-significant differences when adjusted for age and sex (Table 7). The results of sequencing confirmed the accuracy of the PCR assay. The sequence of all obtained amplicons was 91%–99% identical to the corresponding GenBank sequence.

Discussion

The composition of the intestinal microflora is unique but it is stable for each individual.²⁵ It has a critical role in modulating the immune response of the gut as well as the initiation and continuation of IBD. In healthy individuals, the protective cell-mediated and humoral immune responses against enteropathogenic microorganisms were allowed to progress, while responses to normal microflora were prevented.²⁶ This homeostasis might be disrupted. Then the commensal flora could act as a replacement bacterial pathogen, and because the host response is unable to eliminate the flora, the inflammation in IBD occurs.²⁷

Previous culture and molecular studies showed that a dysbiosis might occur in anaerobic bacteria populations in UC patients.²⁸ Specific PCR primers were selected to cover

Table 4. Numbers and Percentages of Aerobic and Facultative-anaerobic Bacteria Identified

	<i>E. coli</i> No. (%)	<i>Klebsiella</i> spp. No. (%)	<i>Proteus</i> spp. No. (%)	<i>Staphylococcus</i> spp. No. (%)	<i>Streptococcus</i> spp. No. (%)	<i>Enterococcus</i> spp. No. (%)
UC samples	12 (30.0%)	9 (22.5%)	5 (12.5%)	5 (12.5%)	4 (10.0%)	2 (5.0%)
Non-UC samples	9 (22.5%)	12 (30.0%)	10 (25.0%)	5 (12.5%)	3 (7.5%)	2 (5.0%)
OR (95% CI)	1.47 (0.54 – 4.02)	0.67 (0.24–1.84)	0.42 (0.130 – 1.39)	1.00 (0.26- 3.76)	1.37 (0.28 – 6.55)	1.00 (0.13 – 7.47)
<i>P</i> < 0.05	0.446	0.446	0.152	1.000	0.692	1.000

Table 5. Logistic Regression of Association Between Aerobic and Facultative-Anaerobic Bacterial Species and UC^a

Aerobic Bacterial Species	Adjusting OR	(95% CI)	<i>P</i> Value
<i>E. coli</i>	1.807	0.613–5.325	0.283
<i>Klebsiella</i> spp.	0.742	0.258–2.131	0.579
<i>Proteus</i> spp.	0.390	0.110–1.381	0.145
<i>Staphylococcus</i> spp.	0.970	0.245–3.845	0.966
<i>Streptococcus</i> spp.	1.082	0.210–5.567	0.925
<i>Enterococcus</i> spp.	0.713	0.090–5.655	0.749

^a Adjusted OR for age and sex

an extensive range of bowel bacteria at group, genus, and species levels. By using DNA-based molecular techniques in this research, the obvious difference was seen only in *F. prausnitzii*, *Prevotella* and *P. productus* in UC and non-UC patients (a significant reduction was observed).

Faecalibacterium prausnitzii is an important commensal bacterium and one of the abundant anaerobic species in the gastrointestinal tract of humans and accounts for around 8% of the total bacterial population in the colon.²⁹ Moreover, it plays an essential role in maintaining intestinal health.³⁰ *F. prausnitzii*, that were first classified as *F. prausnitzii*, were more closely related to members of *Clostridium cluster IV* (the *Clostridium leptum* group).³¹ The main action of these bacteria is to provide energy.³² The major product of glucose fermentation by *F. prausnitzii* is butyrate.³³ This substance plays a major role in gut physiology, protection against pathogen invasion, and modulation of the immune system.³⁴ On the other hand, it is responsible for inhibition of histone deacetylase activity.³⁵ Therefore, butyrate may contribute to the anti-inflammatory effect and might lead to the alleviation of oxidative stress in the epithelial layer of the gut followed by inhibition of the growth of potential pathogens such as *E. coli*.³⁶

In contrast, pathogenic *Enterobacteriaceae*-induced inflammation represses the growth of *F. prausnitzii* close to the mucus layer by creating oxidative stress.³⁷ The majority of recent studies suggest that changes in the population of *F. prausnitzii* might be related to different human disorders such as CD, active UC, and alternating-type irritable bowel syndrome (IBS-A).³⁰ Our observation is in agreement with the study by Machiels et al.³⁸ They found that the number of *F. prausnitzii* bacteria in UC patients was significantly lower than the control group. Therefore, treatments such as probiotics or prebiotics that increase *F. prausnitzii* levels in patients with IBD might be helpful.³²

Genus *Peptostreptococcus* is a member of the *Clostridiaceae*.

It is dominant in the lower part of the gastrointestinal tract.³⁹ This is only one of the few bacterial populations that can damage intestinal mucin. Overexpression of mucin may result in overgrowth of bacteria and is associated with many types of cancer. Therefore, the presence of this microorganism in the intestine is important. Our results indicated significant differences and low levels of *P. productus* in UC patients, which was in agreement with other research also showing this microorganism was decreased in UC patients.⁴⁰

Prevotella spp. is the most abundant microbial population associated with the colonic mucosa and plays an important role in maintaining the community structure of human gut microbiome.⁴¹ *Prevotella* spp. has an essential role in the biosynthesis of vitamin B1⁴² and play an essential role in digesting carbohydrate-rich food.⁴³ In this study, a decrease in the population of *Prevotella* in UC patients was found, which is in agreement with some other studies. Although, some other studies have shown that the population of this bacterium in colon biopsy specimens from patients with UC was higher than in controls.²⁶

The results of this investigation help to understand variations in the bacterial flora among UC and non-UC patients. Indeed, these data support the hypothesis that the composition of the intestinal microflora is associated with induction, continuation, and reactivation of IBD through interaction with the host, which produces inappropriate immune reactions and results in uncontrolled inflammation and dysbiosis. Imbalance of the gut ecosystem leads to abnormal reactivity of the mucosal immune system against enteric anaerobic bacteria. Moreover, it may result in a change in butyrate production, intestinal mucin, biosynthesis of vitamin B1, and degradation of polysaccharides. This underscores the importance of unbalanced microbiota in etiology of IBD.

In conclusion, our study presented a comprehensive

Table 6. Number and Percentage of UC and Non-UC Groups With Positive Polymerase Chain Reaction for Anaerobic Bacterial spp.

Anaerobic Bacterial Species	UC Samples No. (%)	Non-UC Samples No. (%)	OR (95% CI)	P Value
<i>Bifidobacterium</i> spp.	26 (65.0)	19 (47.5)	2.05 (0.83 – 5.04)	0.115
<i>B. adolescentis</i>	22 (55.0)	30 (75.0)	0.40 (0.15 – 1.05)	0.061
<i>B. longum</i>	33 (82.5)	27 (67.5)	2.27 (0.79 – 6.48)	0.121
<i>Prevotella</i> spp	17 (42.5)	27 (67.5)	0.356 (0.14 – 0.88)	0.025*
<i>Bacteroides fragilis</i> group	26 (65.0)	24 (60.0)	0.75 (0.29 – 1.97)	0.644
<i>B. thetaiotaomicron</i>	30 (75.0)	27 (67.5)	1.44 (0.54 – 3.82)	0.459
<i>B. distasonis</i>	5 (12.5)	1 (2.5)	5.57 (0.62 – 50.03)	0.090
<i>B. vulgatus</i>	26 (65.0)	28 (70.0)	0.79 (0.31 v 2.03)	0.633
<i>E. bifforme</i>	1 (2.5)	0 (0)	-	0.5
<i>F. prausnitzii</i>	24 (60.0)	36 (90.0)	0.16 (0.05 – 0.56)	0.002**
<i>P. productus</i>	26 (65.0)	34 (85.0)	0.32 (0.11 – 0.96)	0.039*
<i>Lactobacilli</i>	18 (45.0)	19 (47.5)	0.90 (0.904 – 2.18)	0.823
<i>Clostridium butyricum</i>	28 (70.0)	22 (55.0)	1.90 (0.76 – 4.78)	0.166

UC = ulcerative colitis; %, percentage of anaerobic bacterial strains isolated in colon mucosa biopsy specimens calculated as number of isolates found with respect to total bacterial isolates detected in each group, OR (95% CI), odds ratio (95% confidence interval), and, $P < 0.05$.

* Significant difference, ** high significant difference.

Table 7. Logistic Regression of Association Between Anaerobic Bacterial Species and UC^a

Anaerobic Bacterial Species	Adjusting OR	95% CI	P Value
<i>Bifidobacterium</i> spp.	2.241	0.850 – 5.910	0.103
<i>B. adolescentis</i>	0.417	0.154 – 1.130	0.086
<i>B. longum</i>	2.018	0.673 – 6.054	0.210
<i>Prevotella</i> spp	0.425	0.158 – 1.146	0.091
<i>Bacteroides fragilis</i> group	0.730	0.280 – 1.905	0.520
<i>B. thetaiotaomicron</i>	1.736	0.614 – 4.913	0.298
<i>B. distasonis</i>	5.703	0.588 – 55.286	0.133
<i>B. distasonis</i>	0.999	0.372 – 2.686	0.999
<i>B. vulgatus</i>	-	-	-
<i>E. biforme</i>	0.166	0.046 – 0.594	0.006 ^b
<i>F. prausnitzii</i>	0.285	0.090 – 0.910	0.034 ^c
<i>Lactobacilli</i>	1.003	0.397 – 2.534	0.994
<i>Clostridium butyricum</i>	1.509	0.575 – 3.960	0.403

Abbreviations: UC, ulcerative colitis; OR, odds ratio.

^a Adjusted OR for age and sex

^b High significant difference.

^c Significant difference.

analysis of mucosal microbiota in the gut of Iranian patients with UC and suggested a possible protective benefit of *F. prausnitzii*, *Prevotella*, and *P. productus* against the development of UC. Therefore, treatment with probiotics or prebiotics which increase the levels of *F. prausnitzii*, *Prevotella*, and *P. productus* in UC may help to maintain a normal intestinal microbiota in the gut. However, these results are preliminary and confirmation by further studies is required.

Authors' Contribution

LA is the first author who carried out all laboratory experiments, collected data and drafted the manuscript. SM is the gastroenterologists who conducted colonoscopies and provided the specimens from all cases. LA participated in the collection of the specimens. AB participated in statistical analysis. BN has supervised all parts of the study. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors have no conflicts of interest.

Ethical Statement

This study was approved by the ethics committee of Ministry of Science, Research and Technology, University of Tehran, Iran (permission no: 27211.6.5). All participants provided written informed consent for the biopsies taken to conduct this study.

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