Gut Dysbiosis Is Related With Activity And Remission Phases Of Ulcerative Colitis And Healthy Condition

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Research

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Abstract

Background. Ulcerative Colitis (UC) is a frequent type of Inflammatory Bowel Disease, characterized by periods of remission and exacerbation. Gut dysbiosis may influence pathophysiology and clinical response in UC. The purpose of this study was to evaluate whether gut microbiota is related to the active and remission phases of UC compared to healthy subjects.

Results. Cross-sectional study. Fecal samples from 18 patients with UC (clinically characterized as active (n=9), remission (n=9)) and 15 healthy subjects were collected. After fecal DNA extraction, the 16S rRNA gene was amplified and sequenced (Illumina MiSeq platform), operational taxonomic units were analyzed with the QIIME (Quantitative Insights Into Microbial Ecology) software. Alpha and beta diversities were compared between clinical settings, as well as the relation between most frequent genus with UC severity indicators. Gut microbiota composition revealed higher abundance of the phyla Proteobacteria and Fusobacteria in active UC, as compared with remission UC and healthy subjects. Likewise, marked abundance of the genus Bilophila and Fusobacteria were present in active UC, as compared with the other groups, whereas higher abundance of Faecalibacterium characterized both remission UC and healthy subjects. Microbial community’s richness and diversity in active UC were significantly different from the other groups. Relative abundance of Fecalibacterium and Roseburia showed higher correlation with fecal calprotectin, while relative abundance of Bilophila and Fusobacterium showed AUCs (Area under the curve) 0.917 and 0.988 for active vs remission UC, respectively.

Conclusion. Gut dysbiosis is related to clinically relevant phases of UC and healthy controls. Particularly, Fecalibacterium, Roseburia, Bilophila, and Fusobacterium were identified as genus highly related with clinical phases of UC.

Introduction

The intestinal tract houses a large and diverse community of microorganisms, collectively referred to as the gut microbiota. These microorganisms participate in human health, by promoting both immune and metabolic functions [1]. It is widely accepted that the gut microbiota has a crucial role in regulating the function of the intestinal epithelium, the immune system and its homeostasis within the gut [2]. The term “dysbiosis” refers to an unbalance in the composition and function of the microbiota [3, 4]; whereas gut dysbiosis, along with altered host’s immune response, have been observed in clinically relevant immunological and inflammatory diseases, such as Ulcerative Colitis (UC) that is a frequent type of Inflammatory Bowel Disease, characterized by periods of remission and exacerbation [3]. Gut microbiota from patients with UC shows a reduced number of bacteria with anti-inflammatory capacities and a higher proportion of bacteria with pro-inflammatory properties. Microbiota diversity is reduced as well, and abundance of microorganisms like Firmicutes has been found low and abundance of Proteobacteria is high [5, 6]. Although a causal effect has not been evidenced; nowadays, it is widely accepted that altered interactions between gut dysbiosis and the intestinal immune system promote UC [2], while the
precise nature of the intestinal microbiota dysfunction in UC remains to be elucidated. Gut microbiota has been considered as a “fingerprint” reflecting the natural history of UC, since it associates with the clinical severity, remission and flare-up responses [5, 6]. Hence, the recognition of such dysbiotic signatures may be useful for an early identification of clinical-therapeutic phases of UC. In this sense, gut microbiota characterization has been largely facilitated by the rapid development and application of culture-independent, high throughput DNA based sequencing technologies, notably those targeting the 16S ribosomal RNA (rRNA) gene [7]. Given the increasing UC prevalence worldwide, including Latin American countries [8, 9], along with the strong interest to understand the relation of a dysbiotic gut microbiota with clinically relevant phases of UC and their early identification, the present study aimed to characterize gut microbiota as related with UC’s clinical phases, using control comparison with healthy subjects.

Materials And Methods

Study population

In this cross-sectional study, groups of 9 patients each (active UC, remission UC subjects who attended to the Department of Gastroenterology, National Medical Center ‘20 de Noviembre’ ISSSTE, Mexico City, Mexico, between July 2017 and January 2019) and 15 control healthy subjects were evaluated. Patients with concomitant irritable bowel syndrome, pseudomembranous colitis, and antibiotic treatment during the previous 4 weeks were excluded. UC was defined according to clinical, radiological, endoscopic, and histological criteria [10, 11]. The study population with UC received therapy based on pharmacological treatment, a diet rich in fiber, and the use of probiotics. Characteristics like age, time since initiation of the disease, affected gastrointestinal localization, frequency of bowel movements, and presence of blood in stool were collected from clinical records. Active UC was defined as a value of 4 or higher for colitis activity index (CAI (Clinical activity index), used for ulcerative colitis). Clinical remission was defined by the validated CAI, and it was considered when the scores maintained lower values for at least 3 months [12]. Healthy subjects were volunteers without previous history of chronic disease, owning to a different family from those with UC, but with a similar diet.

Ethical statement

The study was carried out according to the 1975 ethical guidelines of the Declaration of Helsinki. All participants provided written informed consent. The study was approved by the Local Committees of Research, Ethics in Research and Biosafety of the Centro Médico Nacional ‘20 de Noviembre’ ISSSTE, Mexico City (Protocol ID No. 358.2017).

Stool samples

Stool samples were collected either during hospitalization (active UC) or prepared at home and collected during programmed medical consultation (remission UC and healthy subjects; samples were stored at
home between 4°C and 8°C for up to 24 hours, before hospital collection). Samples were collected with the help of a stool sampling kit, which consisted of a plastic lining to cover the toilet, two stool sample tubes with spoons, two plastic bags, and a clipping system for safe closure of the outer bag. Samples were labeled upon arrival, and one part was processed for fecal calprotectin assay; while the remaining was aliquoted and frozen directly at −80 °C for further microbiota analyses [12, 13].

**DNA extraction of fecal samples**

Frozen stool samples were thawed on ice, and approximately 200 mg were added to dry bead tubes with lysis buffer (AllPrep PowerFecal DNA, Qiagen). The stool samples were homogenized followed by a combined chemical and mechanical lysis by using prefilled lysis tubes. Inhibitors commonly present in stool samples were then removed before isolation of nucleic acids. DNA isolation was continued by using the AllPrep DNA MiniElute spin column, according to the manufacturer's instructions. DNA was eluted in 30μL EB-buffer. Negative control samples (consisted in only PCR grade water) were handled in exactly the same way as the fecal samples, in order to rule out contamination during the isolation procedure [14]. A Nanodrop ND-1000 (NanoDrop Technologies, USA), was used to estimate DNA concentrations. DNA concentration was adjusted to a final concentration of 10 ng/ul [15].

**Amplification and sequencing of bacterial 16S rRNA gene**

The V3 and V6 hypervariable regions of the 16S rRNA gene were PCR amplified from microbial genomic DNA with the forward (TATGGTAATT-GT-GTGCCAGCMGC CGCGGTAA) and reverse (GGACTACHVGGGTWTCTAAT) primers. The primers were designed with overhanging adapters (Forward: AATGATA CGGC GACC ACCGA GATCT ACAC), (Reverse: GGACTACHVGGGTWTCTAAT) for annealing to Illumina universal index sequencing adaptors that were added in a later PCR [16]. The PCR products were evaluated by 2% agarose gel electrophoresis and purified. After purification, spectrophotometry was used to quantify the PCR products. Samples were normalized to a final concentration of 2nM [16, 17].

**Microbial composition and analysis by Illumina**

A 2 steps PCR methodology was used to prepare 16S rRNA libraries. For the first-step, extracted DNA was quantified and samples were diluted to the amount of the least concentrated sample. Then 2μL were used for the PCR reaction (quadruplicates) at the following conditions 98˚C for 30 s [98˚C for 30 s, 52˚C for 30 s, 72˚C for 30 s] for 20 cycles, 4˚C hold. Then, the 4 resulting reactions were amalgamated. The samples were then cleaned by using AmpureXP beads and eluted in 40μL final volume. For the second step, a 4μL of the obtained DNA was mixed with primers PE-PCR-III-F and PE-PCR-IV-barcode, in a 25μL final volume PCR reaction (quadruplicates), at run cycle conditions of 98˚C for 30 s [98˚C for 30 s, 83˚C for 30 s, 72˚C for 30 s] for 7 cycles, 4˚C hold. Then, the 4 PCR reactions were pooled and the products cleaned by using 16S Metagenomic Sequencing Purification beads [18]. The DNA library concentrations were quantified and then multiplexed to provide the same amount of DNA in each sample. A single Illumina MiSeq lane set for paired-end 300-basepair reads was used to sequence the libraries. Paired-end
reads of 16S rRNA gene libraries were generated with the Illumina, MiSeq platform. A total of 10,629,314 raw sequences were obtained, with further quality filter and binned resulting in 8,349,697 usable sequences, with a sample average of 378,489 per sequence. Sequences were clustered and singletons removed; the data were rarefied to control for variations in sequencing efforts. The datasets supporting the conclusions of this article are available in the https://www.ncbi.nlm.nih.gov/bioproject/596546, under the ID PRJNA596549 repository. The analyses of taxonomy and diversity of the samples were performed taking as a reference the SILVA database.

Bioinformatic Analysis

Illumina Real-Time Analysis software (version 1.17.28) was used for base calling, image analysis, and error estimation. Sequencing provided read lengths of 300 bp, which were demultiplexed and verified that the paired ends provided a clear overlap. The paired ends were then linked together with the fastq-join program (http://code.google.com/p/ea-utils/). Separate files of each sample (R1 and R2) were entered in fastq format by using the split_libraries_fastq.py pipelines. Sequences that had quality value (QV) scores of \( \geq 20 \) (Phred score of 20) for no-less than 99% of the sequence were selected for further study [19]. All sequences with ambiguous base calls were discarded. Subsequently, the sequences were grouped in Operational Taxonomic Units (OTU) where the pick_closed_reference_otus.py pipelines were used. QIIME which uses the BIOM format, was used to represent OTU tables [16, 20, 21]. Analyses of sequence reads were performed by using SILVA multiclassifier tools with a 97 % confidence threshold [22]. Subsequent analyses of diversity index were all performed based on this output normalized data [23]. To perform the diversity analyses, the core_diversity_analyses.py pipelines were executed with the pipeline alpha_diversity.py. Alpha diversity metrics were calculated with QIIME, the observed OTUs (observed species) and the phylogenetic diversity or complete tree PD (PD_whole_tree) [21]; whereas the weighted distances of UniFrac of the beta diversity was determined with beta_diversity.py pipelines and R software v.2.15.3 was used to display the results [24].

Fecal calprotectin test

Fecal calprotectin (FC) was measured as a marker of intestinal inflammation by using a commercial ELISA (MyBioSource, California, San Diego), following the manufacturer’s instructions. Optical densities were read at 405 nm with a microplate ELISA reader. Samples were tested in duplicate, and results were calculated from a standard curve and expressed as \( \mu g/g \) stool [25].

Statistical analysis

Data normal distribution was evaluated with Shapiro–Wilk Test. Quantitative data were compared by non-paired, 2-tail, T-test or U-Mann Whithney, as appropriate. Statistical analyses of the sequences were carried out in QIIME and R. Multivariate non-parametric ANOVA (Analysis of Variance) was used to
determine the differences in the abundance of the microbial community between groups, whereas Spearman's correlation was used to determine the relation between specific microbiota abundance and fecal calprotectin concentration. In order to test whether the clusters of microbiota from the study conditions were different between them, UniFrac P values, based on principal coordinate analysis applied to the matrix distance, was performed to allow pairwise comparison of microbiota from clinical phases of UC and healthy controls [26,27]. Finally, we determined the area under the curve to evaluate whether the relative abundance of the bacterial genus most frequently observed (cutoff value according to ROC analysis) may be related with UC severity. The Statistical Package for Social Sciences SPSS v.18.0. was used, and P values of $\leq 0.05$ (2-tailed) were considered to be statistically significant.

Results

Study population

Eighteen patients diagnosed with UC, mean aged 37 years old constituted the study population, which were further divided according to their UC severity, as demonstrated by the CAI and fecal calprotectin values. A cohort of sex- and age-matched, healthy volunteers was included for comparison. Baseline clinical-demographic characteristics are shown in Table 1.
Table 1
Demographic and clinical characteristics of the study population (n = 33)

|                        | UC Active (n = 9) | UC Remission (n = 9) | Healthy subjects (n = 15) | \( p \) value |
|------------------------|------------------|----------------------|---------------------------|--------------|
| Age (years old)        | 36.9 ± 1.4       | 37.9 ± 1.1           | 36.4 ± 1.6                 | NS           |
| Male                   | 7 (70)           | 6 (60)               | 6 (60)                     | NS           |
| Index CAI              | 11.0 ± 1.3       | 1.7 ± 0.6            | N/A                       | < 0.05       |
| Montreal A (age at onset) | none            | none                 | N/A                       | NS           |
| A1 (16)                | 7 (70)           | 6 (60)               |                           |              |
| A2 (17–40)             | 2 (20)           | 3 (30)               |                           |              |
| A3 (41)                |                  |                      |                           |              |
| Montreal Score         | 1 (10)           | none                 | N/A                       | NS           |
| E1 ulcerative proctitis| none             | 4 (40)               |                           |              |
| E2 left sided UC       | 8 (80)           | 5 (50)               |                           |              |
| E3 extensive UC        |                  |                      |                           |              |
| Endoscopy Mayo Score   | none             | N/A                  | N/A                       | N/A          |
| 0                      |                  |                      |                           |              |
| 1                      | 1 (10)           |                      |                           |              |
| 2                      | 8 (80)           |                      |                           |              |
| 3                      |                  |                      |                           |              |
| Frequency of bowel movements | ≥ 10         | 4 to 6               | 1 to 2                     | NS           |
| Presence of blood in stool | 8 (80)       | None                 | None                      | NS           |
| Time (years) from diagnosis | 8 (80)       | 6 (60)               | N/A                       | NS           |
| ≥ 10                   | 1 (10)           | 3 (30)               |                           |              |
| ≤ 10                   |                  |                      |                           |              |

Quantitative data was resumed as mean ± SD and qualitative data as n (%). Statistical analysis was performed with 2-way U-Mann Whitney and Fisher test, as appropriate. Abbreviations: UC, Ulcerative Colitis; N/A, not applicable; NS, non-significant.
|                           | UC Active (n = 9) | UC Remission (n = 9) | Healthy subjects (n = 15) | p value |
|---------------------------|------------------|----------------------|---------------------------|---------|
| Currently smoking         | 2 (20)           | None                 | none                      | N/A     |
| Medication use            | none             | 6 (60)               | N/A                       | NS      |
| Mesalazine                | 2 (20)           | 2 (20)               |                           |         |
| Corticosteroids           | none             | 1 (10)               |                           |         |
| Infliximab                | 7 (70)           | None                 |                           |         |
| No treatment              |                  |                      |                           |         |
| Fecal calprotectin (µg/g) | 239.3            | 111.2                | 30.2                      | p < 0.05|

Quantitative data was resumed as mean ± SD and qualitative data as n (%). Statistical analysis was performed with 2-way U-Mann Whitney and Fisher test, as appropriate. Abbreviations: UC, Ulcerative Colitis; N/A, not applicable; NS, non-significant.

**Microbial composition and diversity**

The analysis of microbiome from fecal samples showed the relative abundance of OTUs at different taxonomic levels (Figs. 1A and 1B, Table 2). OTUs were created out of the filtered tags and were grouped at a similarity of 97%. This gave a total of 1533 OTUs for the 30 samples used in this study. Taxonomic composition at the level of phyla is summarized in Fig. 1A. The bacterial phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria* were the most common sequences showing 97% of similarity. For remission UC and healthy subjects, *Firmicutes* was the most abundant bacterial phylum. Microbiota abundance in remission UC was very similar to that observed in healthy subjects, at the phyla level, whereas, active UC showed phylum *Proteobacteria* as the most abundant. Genus distribution provided a subjective perception of difference between the relative abundance of patients with UC active vs UC remission and healthy subjects (Fig. 1B). The most abundant genus in active UC were *Fusobacterium* and *Bilophila*, followed by *Bacteroides*, *Escherichia*, *Enterococcus*, *Pseudomonas*, *Ruminococcus*, *Aeromonas*, *Veillonella*, and *Acinetobacter*. For the group of remission UC and healthy subjects, the most abundant genus were *Faecalibacterium*, *Roseburia*, *Bacteroides*, *Prevotella*, *Escherichia*, *Eubacterium*, *Akkermansia*, *Ruminococcus*, *Lactobacillus*, and *Butyricimonas*.

Interestingly, the relative abundance of most frequent bacterial genus observed in active UC was significantly different from those corresponding to remission UC and healthy subjects (Fig. 2). For comparative purposes, data obtained in external cohorts from published studies [28, 29, 30] were included (Table 2, lower panel). Our findings of relative abundance of gut microbiota were similar to those
observed by Franzosa *et al.*, Kumari *et al.*, and Sha *et al.* regarding active, remission UCs and healthy subjects.
Table 2
Comparison of gut dysbiosis in fecal samples from UC population

| Most abundant gut microbiota in our study | Healthy subjects (n = 15) | Active UC (n = 9) | Remission UC (n = 9) |
|------------------------------------------|--------------------------|------------------|---------------------|
| Phylum                                   |                          |                  |                     |
| Firmicutes                               | 54.6 ± 6.4               | 4.0 ± 1.5 **     | 50.0 ± 5.2          |
| Bacteroidetes                            | 45.0 ± 3.4               | 15.0 ± 0.2 **    | 46.0 ± 4.2          |
| Proteobacteria                           | 2.5 ± 1.0                | 52.5 ± 5.6 **    | 0.0 ± 0.0           |
| Fusobacteria                             | 0.0 ± 0.0                | 30.0 ± 2.5 **    | 0.0 ± 0.0           |
| Actinobacteria                           | 2.5 ± 1.0                | 1.5 ± 0.5        | 2.5 ± 1.0           |
| Verrucomicrobia                          | 1.5 ± 0.5                | 0.0 ± 0.0        | 1.5 ± 0.3           |
| Genus                                    |                          |                  |                     |
| Lactobacillus                            | 8.5 ± 2.4                | 0.0 ± 0.0 **     | 5.6 ± 4.2           |
| Faecalibacterium                         | 40.2 ± 4.9               | 0.5 ± 1.5 **     | 21.0 ± 8.7 ***      |
| Roseburia                                | 7.3 ± 7.4                | 0.0 ± 0.0 **     | 5.4 ± 7.2 ***       |
| Bacteroides                              | 3.5 ± 2.1                | 7.6 ± 4.1        | 11.5 ± 10.8 ***     |
| Bilophila                                | 0.0 ± 0.0                | 12.0 ± 9.1 **    | 0.0 ± 0.0           |
| Fusobacterium                            | 0.0 ± 0.0                | 35.6 ± 15.4 **   | 0.0 ± 0.0           |

| Most abundant gut microbiota in others studies | Healthy subjects (n = 20) | Active UC (n = 20) | Remission UC (n = 22) |
|-----------------------------------------------|---------------------------|--------------------|-----------------------|
| Genus                                         |                           |                    |                       |
| Lactobacillus                                 | 56.0 ± 12.0               | 0.010 ± 0.042 **   | 100.1 †               |
| Faecalibacterium                              | 79.0 ± 60.0               | 0.017 ± 0.017 **   | 63.5 †                |
| Roseburia                                     | 47.0 ± 10.0               | 0.039 ± 0.003 **   | 78.8 †                |
| Bacteroides                                   | 9.4 ± 2.1                 | 40.9 ± 25.2 *      | 88.8 †                |
| Bilophila                                     |                           | 33.5 ± 18.3 *      | ND                    |
| Fusobacterium                                 | 0.0 ± 0.0                 |                    | ND                    |

Relative abundance is shown as mean ± SD and (†) percentage of the relative abundance in relation to that observed in healthy subjects. Statistical analysis was performed with 2-way ANOVA. Significant difference (p < 0.01) between: (*) Active UC vs Remission UC; (**) Active UC vs Healthy subjects; (***) Remission UC vs Healthy subjects. In the lower panel, comparison with the relative abundance obtained by other studies is provided (Data sources: results from external cohorts from Franzosa et al., 2019; Kumari et al., 2013 and Sha et al., 2013.) Abbreviations: UC, Ulcerative Colitis.

Regarding bacterial alpha diversity comparison, active UC patients showed the lowest community richness (Chao index) and diversity (Shannon index) (Fig 1C), whereas community richness and diversity...
Discussion

Our main finding was the significant differences of fecal microbiota composition from patients with active UC vs those with remission UC and healthy subjects, with potential clinical application. Our study population was younger aged and most of them at advanced stages of UC. These characteristics are comparable with population from other studies exploring fecal microbiota [2, 28, 31, 32, 33, 71] whereas healthy subjects controls were volunteers from a family with a similar diet, expected to exert lower influence on the gut microbiota composition. Our results showed an increased proportion of the phylum Proteobacteria and the genus Fusobacterium, and Bilophila in active UC, which was significantly different from the group of remission UC and healthy subjects, who shared a microbiota profile of higher proportion of phylum Firmicutes, and genus Faecalibacterium, and Roseburia. These results are comparable with studies carried out by Franzosa et al., 2019, Kumari et al., 2013 and Sha et al., 2013. Particularly, the findings of reduced proportion of genus Lactobacillus, Faecalibacterium, and Roseburia in active UC, and their restoration in remission UC, has also been observed in previous reports [33, 34, 35, 36, 37, 73]. Such characterization is relevant due to scanty information regarding microbiota abundance in remission phase of UC, whereas consistent identification of specific genus in remission UC may be useful design for more efficient therapeutic strategies, prompted to reduce UC severity. Interestingly, particular bacterial composition like Faecalibacterium was shared by remission UC and healthy subjects. These bacteria have been reported to metabolize dietary components that promote colonic motility, maintain intestinal immune system and anti-inflammatory properties [38, 39, 40]. Consistently, reduced abundance of these microorganisms have been associated with a higher rate of recurrence of UC [41, 42, 43, 44, 45, 71] although increased levels of Faecalibacterium in stool samples have been associated with a lower activity index; supporting their role as potential biomarkers of disease severity and outcome, as suggested in other studies [46, 47].
Other findings were the higher abundance of the phylum *Proteobacteria*, and particularly the expansion of the genus *Bilophila*, in active UC. It is known that the relative abundance of *Bilophila* is promoted by diets enriched in saturated fats, which increase bacterial resistance to bile elimination. Furthermore, a change in the type of fat consumed impacts the composition of gut microbiota, which may modify the onset and severity of UC [39, 48,49]. Certain species of *Fusobacterium* show pro-inflammatory, invasive and adherent capacity to the intestinal mucosa, while increased proportion of *Bilophila* in the gut promotes an immune response mediated by Th1, resulting in the development of colitis in experimental mice model [50, 51, 52]. According to data from the present study, as well as those from the comparative studies [28, 29, 30], higher abundance of *Fusobacterium* and *Bilophila* was observed in the group with UC activity, while they tended to disappear in the remission phase.

Although direct pathophysiological mechanism is not possible to elucidate from the present study, we can propose that the relative abundance of some species are associated with the degree of inflammation and UC activity, derived from the inverse relation observed between the abundance of *Fecalibacterium* and *Roseburia* with calprotectin, a biomarker of severity of UC, which was consistent with a recent report [53]. Likewise, differences in bacterial richness, diversity, and dominance were highly related to the clinical scenarios studied. Remarkably, remission UC and healthy subjects showed the highest relative abundance of the phylum *Firmicutes*, which contributed to most of bacterial diversity and richness [54, 55, 56]. Further analysis of cluster distribution of bacterial communities showed differences in active UC, as compared to remission UC and healthy subjects, which was consistent with previous studies showing difference in the structure of microbiota between UC and healthy subjects [57, 58, 59].

Furthermore, studies characterizing gut microbiota composition and its modification during UC are relevant, since: a) UC provides a higher risk for colorectal cancer, whereas gut dysbiosis is thought to facilitate colorectal cancer development; b) the study of gut microbial communities during clinical phases of UC contributes to a better understanding of potential interactions with host immune response; c) characterization of specific genus of gut microbial communities may own potential clinical application derived from their association with active or remission phases of UC; and d) specific microbial manipulation, concomitant to antibiotic use, is currently used as a therapeutic approach for UC [49, 60, 61].

Finally, gut dysbiosis has been proposed as an important contributing factor to the increasing prevalence of UC, with a potential role for the related clinical-therapeutic phases [32, 62, 63]. Consistently, we found a significant ability of the genus *Bilophila* and *Fusobacterium* to selectively associate with cases of UC activity/remission. This agrees with the literature that describes a functional role for these bacteria in UC [64, 65, 66, 67] and suggests its potential clinical benefit for an early identification of clinical-therapeutic phases of UC [68, 69].

To our knowledge, this is the first study that investigated the composition of fecal microbiota in Mexican patients with active and remission UC. Our study faces some limitations. First, *16S rRNA* analysis provides the taxonomic composition of the microbes present in the community and does not provide an
analysis of the role of the microbiota in the disease. Second, data analysis may show limitations regarding the specific characterization of microbiota composition, as an isolated endpoint; however, we think that the analysis performed yields to an adequate interpretation within a translational context, highlighting the role of microbiota diversity in the clinical phases of UC. Third, a larger sample size may be required to confirm our data and further research is required to better characterize the role of gut microbiota in UC patients.

Here we provide a broad investigation of the fecal microbial community in Mexican patients presenting UC. We demonstrate differences in the microbiota communities in patients with active UC, remission UC, and healthy subjects. Selective association of gut dysbiosis with UC activity/remission may set the bases for further applications of non-invasive methods, clinically useful for an early identification of disease severity.

**Abbreviations**

ANOVA: Analysis of variance

AUC: Area under the curve

CAI: Clinical activity index

FC: Fecal calprotectin

OTU: Operational Taxonomic Units

QIIME: Quantitative Insights Into Microbial Ecology

QV: quality value

UC: Ulcerative Colitis

**Declarations**

**Ethics approval and consent to participate**

The study was approved by the Local Committees of Research, Ethics in Research and Biosafety of the Centro Médico Nacional ‘20 de Noviembre’ ISSSTE, Mexico City (Protocol ID No. 358.2017).

**Consent for publication**

Not applicable
Availability of data and materials

Sequence data of the study has been submitted to National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/bioproject/596546) under the Accession Number PRJNA596549.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Not applicable

Authors' contributions

The study was conceived by BMA, JASC, and GCE designed the study; analyzed and interpreted the data and results; SJS, JRS, SLAE, RPC de V, CLC, JSGV, TCE, PMT, JS, CHC gave a major contribution to the writing of the manuscript. A native English speaker, JS edited the manuscript for language. BMA, JASC and GCE contributed to experimental design and manuscript production. All authors contributed to the discussion of results and approved the version of the manuscript that was submitted.

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**Figures**
Figure 1

Characteristics of the microbial community in active UC, remission UC and healthy subjects. A) Taxonomic composition distribution in samples of phylum level. B) The taxonomic composition distribution in samples of genus level. C) Alpha diversity index boxplot, including community richness (Chao), D) diversity (Shannon), and E) Dominance (Simpson). p-value indicates statistical significance of 2-way ANOVA.
Abundance analyses. Whisker-box plots comparing bacterial genera in fecal microbiota of active UC, remission UC and healthy subjects. Only the 4 most relevant bacterial genera, according to abundant taxonomic composition, were analyzed: A) Fusobacterium; B) Bilophila; C) Faecalibacterium, and D) Roseburia. p-value indicates statistical significance of 2-way ANOVA.
Figure 3

Gut microbiota abundance and UC severity marker. The plots show the correlation between bacterial genera in fecal microbiota with calprotectin, a biomarker of UC severity. The correlation of the 4 most abundant bacterial genera, according to abundant taxonomic composition: A) Fusobacterium; B) Bilophila; C) Faecalibacterium, and D) Roseburia; were analyzed in the subgroups of active UC (●), remission UC (●), and healthy subjects (●). p-value indicates statistical significance of 2-way, Spearman correlation.
Figure 4

Principal component analysis. The overall structure of the fecal microbiota was plotted according to the different clinical scenarios. Each data point represents an individual sample.