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***Basic Study***

**Fecal microbiota in pouchitis and ulcerative colitis**

Li KY *et al*. Fecal microbiota in pouchitis and UC

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**Abstract**

***AIM***

To investigate the changes in microbiota in feces of patients with ulcerative colitis (UC) and pouchitis by genomic technology.

***METHODS***

Fecal samples were obtained from UC patients with or without ileal pouch-anal anastomosis (IPAA) procedure, as well as healthy persons. Touchdown polymerase chain reaction technique was chose to amplify the whole V3 region of 16S rRNA which was transcribed from deoxyribose nucleic acid extraction in fecal samples. Denaturing gradient gel electrophoresis technique was employed to separate amplicons. The band profiles and similarity indices were digitally analyzed. The predominant microbiota in different groups was confirmed by sequencing the 16S rRNA gene.

***RESULTS***

Microbial biodiversity in healthy people was significantly greater compared with UC groups (*P <* 0.001) and IPAA groups (*P <* 0.001). In comparation with healthy controls, UC patients in remission and mildly active stage, the predominant species of moderately and severely active UC patients changed obviously. Besides, the proportion of the dominant microbiota, which was negatively correlated with disease activity of UC (*r* = -6.591, *P <* 0.01), was decreased in pouchitis. Two kinds of bacteria, *Faecalibacterium prausnitzii* and *Eubacterium rectale,* were detected fewer in number in UC. Patients with pouchitis have altered composition of microbiota compared with UC patients. Microbiota from pouchitis was fewer than that from severely active UC patients. Sequencing results showed similar microbiota such as *Clostridium perfringens* (*C. perfringens*) were shared in both UC and pouchitis.

***CONCLUSION***

Less diverse fecal microbiota was present in patients with UC and pouchitis. Increase of *C.* *perfringens* in fecal may play a role in exacerbation of UC and pouchitis.

**Key words**: Pouchitis; Intestinal flora; Ulcerative colitis; Disease activity index; Ileal pouch-anal anastomosis

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**Core tip:** Dysbiosis occuring in pouchitis might be similar to that observed in ulcerative colitis (UC). This research was designed to determine the altered microflora in patients with UC and pouchitis, and to investigate the relationship between them. Our study demonstrated the reduced biodiversity of fecal microbiota in UC and pouchitis patients. The results showed altered composition of intestinal microbiota in UC and pouchitis, including decreased number of two kinds of commonly seen bacteria in UC, and higher quantity of *Clostridium perfringens* in both UC and pouchitis. The increase of this bacterium in feces may play a role in exacerbation of UC and pouchitis.

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# INTRODUCTION

One of the most common complications in ulcerative colitis (UC) patients operated with ileal pouch-anal anastomosis (IPAA) surgery is pouchitis[1]. Interestingly, it is rarely seen in postoperative patients attacked familial adenomatous polyposis. Gut microbiome plays a vital role in UC[2]. Antibiotics and probiotics are used to treat and prevent pouchitis[3]. The microbiome in UC gut may play a vital role in UC pathogenesis[4].

However, direct evidence of the role of microflora in pathogenesis of pouchitis is lacking. Studies have shown variation in microbiota in pouchitis and healthy controls and no consensus was available based on different culture methods and molecular biology techniques[3]. Johnson *et al*[5] and Lim *et al*[6] showed no differences between pouchitis and no pouchitis (NP) groups. Some studies suggest a reduction in bacterial diversity in pouchitis but not dysbiosis[7]. Some studies reveal an increase in bacterial diversity in pouchitis[8], such as increased numbers of *Clostridium* and *Eubacterium*[9],while others showed less *Enterococcaceae* in pouchitis[10]. The findings of the most recent study reveal that the disorders due to protective and harmful bacteria are associated with pouch inflammation[11]. The emergence of *R. gnavus,* *B. vulgatus* and *Clostridium perfringens (C. perfringens*) and deficiency of *Blautia* and *Roseburia* in patients with UC before IPAA is closely related to pouchitis[12].

Denaturing gradient gel electrophoresis (DGGE) was reported to be used in facilitating the analysis of changes in the composition of intestinal microbiota[2]. We hypothesized that dysbiosis occurring in the pouch might be similar to dysbiosis observed in UC. We determined the altered microflora in pouchitis and UC, and investigated the relationship between them.

# MATERIALS AND METHODS

***Patients and fecal samples***

Patients with IPAA for UC were recruited. Pouchitis was diagnosed based on symptoms, endoscopy and histology of the pouch. Patients underwent pouch endoscopy and biopsy. Physicians recorded clinical data, the pouch appearance and pathological manifestations based on the Pouchitis Disease Activity Index (PDAI)[13]. Antibiotic or other drug therapy was stopped to prevent variations in microbiome 4 wk before collecting the fecal sample. A limited number of patients with pouch were excluded from the study because of antibiotic or probiotic usage for pouchitis or severe concomitant disease.

According to PDAI, patients with IPAA were divided into two groups: NP, PDAI < 7 points (*n =* 11) and pouchitis, PDAI ≥ 7 points (*n =* 8). Matched fecal samples were obtained from healthy people (*n =* 16) and 41 UC patients who did not undergo IPAA. All the UC patients without a pouch underwent endoscopy. Mayo Scoring System for Assessment of Ulcerative Colitis Activity was employed to divide patients with UC into remission group (*n =* 10), mildly activity group (*n =* 11), moderately activity group (*n =* 10) and severely activity group (*n =* 10).

All fecal samples were collected at the hospital and preserved at 4 °C. Upon arrival at the laboratory, the samples were frozen at −80 °C within 12 h. This study was reviewed and approved by Tianjin Medical University General Hospital Ethical Committee (China). Patient data were summarized in Table 1.

***Fecal DNA extraction***

Fecal DNA kit (Aidlab Biotechnologies Co, Ltd, Beijing, China) was used to isolate DNA from frozen feces individually following the manufacturer’s guideline and previously described[14]. Following 1% agarose gel electrophoresis the eluted DNA was quantified on a NanoDrop2000 Spectrophotometer.

***PCR amplification***

The genomic DNA and universal primers including forward and reverse primers (AuGCT DNA technologies, Beijing, China) were employed to amplify the whole fragment V3 region of 16S rRNA gene.

After 15 cycles of thermocycling on a PCR system (Bio-Rad, US) the amplified product was verified by 2 % agarose electrophoresis. The amplified DNA was quantified on a NanoDrop2000 Spectrophotometer, and recorded by DH2000 gel imaging analysis.

***DGGE for amplified 16S rRNA gene***

DGGE was chosen to separate PCR amplicons according to the rules of Muyzer *et al*[15] with some modifications. A 10 % polyacrylamide combined with Tris-acetate-EDTA (TAE) buffer was used for polyacrylamide gels with denaturing gradient at a range from 30 % to 70 %. A stacking gel was added before polymerization of the denaturing gel, followed by appropriate comb insertion. Electrophoresis was performed at 200 V for 5 min and 85 V for 16 h in 0.5 × TAE buffer subsequently at a constant 60 ◦C. After staining with AgNO3[16], the gels were desiccated overnight at 60 ◦C.

***Digital processing of DGGE profiles***

Following the manufacturer's instructions, DGGE profiles were digitally analyzed using Quantity One-4.6.5 in UNIVERSAL HOODⅡ Gel Imaging System (Beijing, China). After normalizing the gels, the band in each sample was marked by the software, and manual corrections were conducted. The number of DGGE bands were shown as mean ± SD. Based on gray value, Dice similarity and UPGMA tree analysis were conducted using Quantity One software. Canoco software was used to conduct Principal Component Analysis (PCA).

***DGGE band extraction and sequencing***

Based on the digital results, the bands distinguishing the groups were purified after excision and sequenced from the gel. Mixture of gel slice and 15 μL TE buffer was heated at 65 °C for 10 min to elute DNA from the gel. The DNA solution was amplified with the universal V3 primers F357+GC clamp and R518. DGGE gel expansion facilitated the purification of the bands. DGGE with an adjusted gradient 32 were used to check the amplicons, which were excised at least three times until a single band was obtained. The DGGE was repeated to purify the PCR product before sequencing. In the final round, the amplicons were analyzed with the original sample profiles from which they were excised and visually analyzed for purification of the correct bands.

When the purified bands matched with the targeted bands, amplicons were chosen to sequence using an ABI Prism. Sequences were read with primers R518 and F357 (without clamp), respectively. BioNumerics software was used for sequencing. GenBank DNA database were searched *via* BLAST homology searches. According to BLAST results, the sequences of phylogenetic neighbour species for reference, whose similarities were up to 90%, were included in cluster analysis using multiple sequence alignments. The purified band sequences were allocated to the most probable species according to the average linking method.

***Statistical analysis***

The Quantity One software was used for Dice similarity analysis and UPGMA tree analysis. DGGE strips bands were presented as mean ± SD. Shapiro–Wilk test was used to test normality of band number and Dice analysis data. The content of each sample was similar. The homogeneity of variance was still robust and highly efficient. The band number of DGGE strips showed normal distribution unlike the Dice analysis results. Therefore, the three groups were tested with SNK test. The Bonferroni test was chosen to compare DGGE strips and band numbers among the five groups. An extension *t* test after non-parametric Kruskal–Wallis *H* test was employed to compare the Dice analysis results among multiple groups. The correlation between disease activity and bacterial count was assessed with Spearman correlation coefficient. SPSS 19.0 software was employed to analyze all the data. Two-tailed tests were used in all analysis and *P* values of ≤ 0.05 were considered statistically significant.

# RESULTS

***Bacteria*** ***in fecal samples from UC***

The demographic details of study patients are seen in Table 1. DNA extracts from the fecalsamples in different individuals presented variable number of bands after PCR-DGGE analysis. A band represents identical or similar sequences of V3 regions of 16S rRNA gene, reflecting the dominant bacterial communities in the fecal samples.

Examination of digital DGGE profiles from healthy controls showed relative stability among the different individuals (Figure 1). Profiles from UC patients showed in Figure 2 suggested significant variation in the position and number of bands when compared with the healthy controls. The number of bands, which reflected diverse microbiota, was 17 ± 3 in the 16 healthy controls and 13 ± 3 in the 41 UC patients (*P* = 0.001). Changes were also seen among the subgroups in UC patients (Figure 3). These results reveal that the number of predominant microbiota was negatively correlated with Mayo classification (*r* = -6.591, *P <* 0.01). Kruskal–Wallis *H* test showed greater similarity between groups than within the groups, which revealed variation in predominant microbiota with clinical status (Table 2). UPGMA tree analysis showed similar conclusion (Figure 4). PCA analysis of normal and UC groups revealed great differences in the predominant species among control group, remission and mild group, and moderate and severe group (Figure 5).

Sequence results after purification (based on digital DGGE profiles) showed the presence of greater number of *C. perfringens* and fewer *Faecalibacterium prausnitzii* and *Eubacterium rectale* in UC than in control group. *C. perfringens* occurred predominantly in severe UC.

***Bacteria in fecal samples of pouchitis***

Significant changes occurred in the position and number of bands from patients with pouchitis when compared with NP and healthy controls (Figure 6). Differences in the number of bands in controls (17 ± 3 bands), NP (11 ± 3 bands) and pouchitis (8 ± 2 bands) are shown in Figure 7 (ANOVA test). Bonferroni test showed greater similarity between groups than within groups, suggesting differences in the predominant species in normal, NP and pouchitis groups (Table3). These results suggest that patients with pouchitis showed altered composition of microbiota compared with normal individuals. UPGMA tree analysis yielded similar conclusions (Figure 8). PCA analysis of normal and UC groups revealed great variation in the predominant species in moderate and severe UC compared with normal, remission and mildly active UC, which also differed from each other (Figure 9).

Sequencing results after purification (based on digital DGGE profiles) showed less *Eubacterium rectale* and more *C*. *perfringens* in pouchitis than in NP and control groups.

As shown in Table 4, the DGGE profiles in pouchitis patients varied significantly from UC in remission to severe state, while NP group of patients differed from UC in remission. The results showed that patients with pouch have altered composition of microbiota compared with UC patients (Figure 10). Microbiota from pouchitis was fewer than in UC patients with a severe condition. A normal pouch can be as diverse as mild, moderate and severe UC. Sequencing results in both UC and pouchitis shared similar microbiota such as *C. perfringens*.

**DISCUSSION**

In this study, we focused on UC patients after IPAA surgery, specifically compared patients developing pouch inflammation with those without surgery. Our digital analysis of stool samples showed that the predominant microbiota in UC patients was reduced when compared with normal group. Sequence analysis showed a higher number of *C. perfringens* and fewer *Faecalibacterium prausnitzii* and *Eubacterium rectale* in the UC group. Levels of*Eubacterium rectale* (butyrate-producing bacteria) group was significantly reduced on UC mucosa[17], and had a high age dependence. High clinical activity indices (CAI), as well as sigmoidoscopy scores (SS), were related with *Eubacterium rectale*[18]*.* Vermeiren considered less *Eubacterium rectale* showed in UC patients *via* a dynamic gut model of mucin environment[17]. *C. perfringens*,which is a kind of Gram-positive, anaerobic, spore-forming bacillus of Clostridia genus, can be found in the intestinal contents of both animals and humans[19]. *C. perfringens* is an intestinal commensal as well as pathogen, for example, *via* production of toxins that damage the host tissues[20]. *C. perfringens* exerts proteolytic and mucinase activity, both of which may mediate the pathogenesis of inflammatory bowel disease (IBD)[21]. *C. perfringens* found in IBD patients, which is deemed to be a important factor during the immunopathogenesis of IBD, may be a result of dysbiosis[22].The results from Falk showed that there were more C. perfringens in pouchitis patients . Some studies found that 21% of the total bacteria in colonic specimens, which were collected from patients attacked UC, belonged to clostridia of clusters I, II and XI, which were not found in the control groups[23]. We should keep in mind that ileum tissues of UC patients were the origin of the present pouches[24]. *F. prausnitzii* is the most species-specific microbe in the study of IBD. Sokol *et al*[25] studied a small group of 17 UC patients and reported a reduction in active UC patients. A strong anti-inflammatory effect of *F. prausnitzii* has been demonstrated both *in vitro* and *in vivo*[26]. Machiels *et al*[27] observed a significant inverse correlation between disease activity, indicating that a deficiency of this species provokes or enhance inflammation. *F prausnitzii* produces high concentrations of butyrate, a vital energy source for colonocytes, and also prevents mucosal atrophy. Consequently, butyrate improves the mucosal barrier function of the colon. Further, butyrate exhibits immunomodulatory and anti-inflammatory effects as it downregulates the pro-inflammatory cytokines[28]. Our data show that bacterial biodiversity in feces was decreased distinctly with the severity of Mayo classification compared with healthy controls. Studies demonstrated that the mucosal biopsies from patients with active Crohn’s disease or active UC manifest reduced bacterial diversity after analysis of 16S rRNA gene[29]. Furthermore, Manichanh[30] reported a reduced phylum Firmicutes in Crohn’s disease in remission using an extensive metagenomic analysis. Consistent with previous studies, our results confirmed that bacterial diversity was reduced in fecal samples from UC patients in different grade, and demonstrated the changes in microbial composition among subgroups in UC. The decreased biodiversity in UC may destroy the stability of gut ecosystem. The results revealed that changes in the predominant bacteria were consistent with Mayo classification. Therefore, we suggest that the fecal microflora in UC patients is reduced in aggravated intestinal lesions. A previous study by Wills[31] reported patient-specific shifts in microbial composition in UC patients with altered pathological activity over time. The changes were more pronounced in CD cases than in UC patients, suggesting their role in the inflammatory process in UC.

On the other hand, DGGE profiles from pouchitis patients varied from UC and healthy controls in the number of bands. We showed a decrease in bacterial diversity and reduced abundance of predominant bacteria in UC pouches. *Ruminococcus* *gnavus* (*R. gnavus*) infection, especially occurring as the predominant microbiota before colectomy, increased the risk of pouchitis 1 year after IPAA[12]. *R. gnavus* produces the bacteriocin ruminococcin A, which inhibits the growth of phylogenetically-related species and various bifidobacterial and clostridial species[32]. The bacteriocin also degraded intestinal mucin[33]. It also induced α-galactosidase and β-glucuronidase activity *in vitro*[34]. β-glucuronidase activity generates toxic metabolites in the colon, which provoke local inflammation. Png *et al*[35] observed an increase of mucolytic bacteria including *R. gnavus* in biopsies of patients with UC and CD. Our data are supported by reports from several groups that analyzed fecal or biopsy samples using different DNA-based methods[7], further confirming the association between changes in microbiota and pouchitis. On the contrary, the variability in endogenous factors, including secretion of mucins, defensins, cytokines, and immunoglobulins, may also affect the composition of predominant bacterial species in UC and pouchits. However, data about these secretions affecting the variability of UC is limited. Studies involving UC have revealed that a high percentage of fecal bacteria (about 30%–40% of dominant species) belong to unusual genus in healthy populations[36]. Hypothetically, the decresed number of stable commensals in individuals, who are genetically susceptible for pouchitis, break this first line of natural defense against potentially invasive bacteria, which result in inflammation.

In conclusion, our research demonstrates the reduced biodiversity of fecal microbiota in UC and pouchitis patients. Fewer numbers of *Faecalibacterium prausnitzii* and *Eubacterium rectale* showed in UC. More *R. gnavus* were found in pouchitis. More *C. perfringens* occurred in both UC and pouchitis.

**COMMENTS**

***Background***

Pouchitis is a commonly-seen complication of patients with ulcerative colitis (UC) following ileal pouch-anal anastomosis (IPAA) procedure. Gut microbiome is considered playing a vital role in the occurrence and development of UC. Besides, antibiotics and probiotics have already been used in treatment and prevention of pouchitis. However, direct evidence of dysbacteriosis in pouchitis is lacking now.

***Research frontiers***

Gut microbiata is hot topic in intestinal inflammation field, especially in inflammatory bowel disease, where data are often conflicting.

***Innovations and Breakthroughs***

The authors demonstrated the role of the reduced diversity and the changed composition of intestinal microbiata in the pathogenesis of pouchitis. We confirmed that *Faecalibacterium prausnitzii* and *Eubacterium rectal* werereduced in UC patients and *Clostridium perfringens* was significantly increased in UC and pouchitis.

***Applications***

The findings provide new clues to better understand the pathogenesis of pouchitis in UC patients operated IPAA, although it won’t be immediately used in treatment of pouchitis.

***Terminology***

**Pouchitis:** It’s an inflammation in intestinal pouch, which is established following proctocoloctomy IPAA procedure for patients with UC to prevent permanent abdominal fistula. It could result in serious consequence, such as blood diarrhea, even ileal pouch failure.

***Peer-review***

The manuscript definitely deals with a hot topic in the inflammatory bowel disease and intestinal inflammation field, where data are often conflicting. The deep insight and detailed description made by the authors of the diversity of microbiota in healthy people and patients will provide new clues to better understand the pathogenesis of pouchitis following IPAA for UC.

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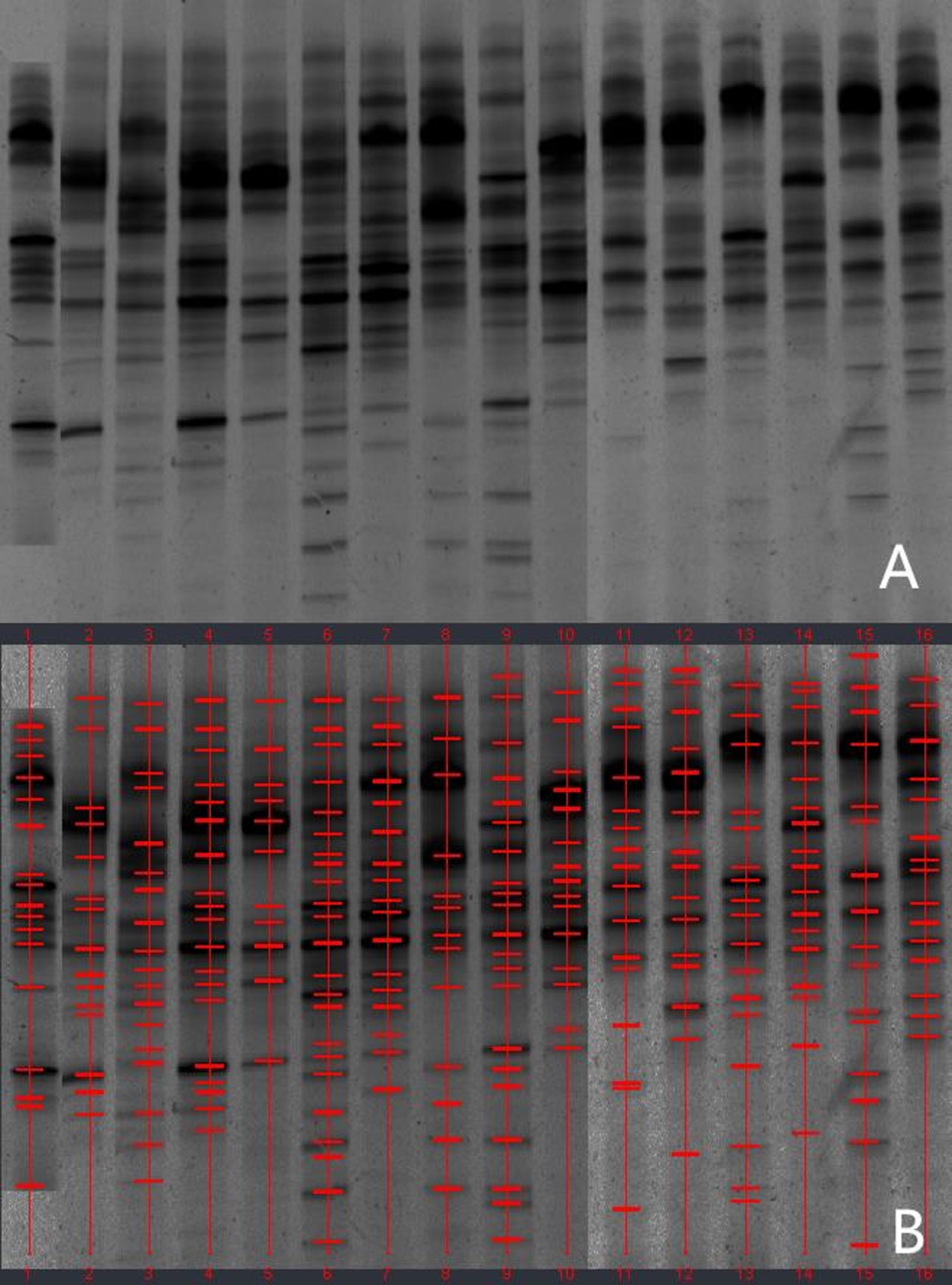
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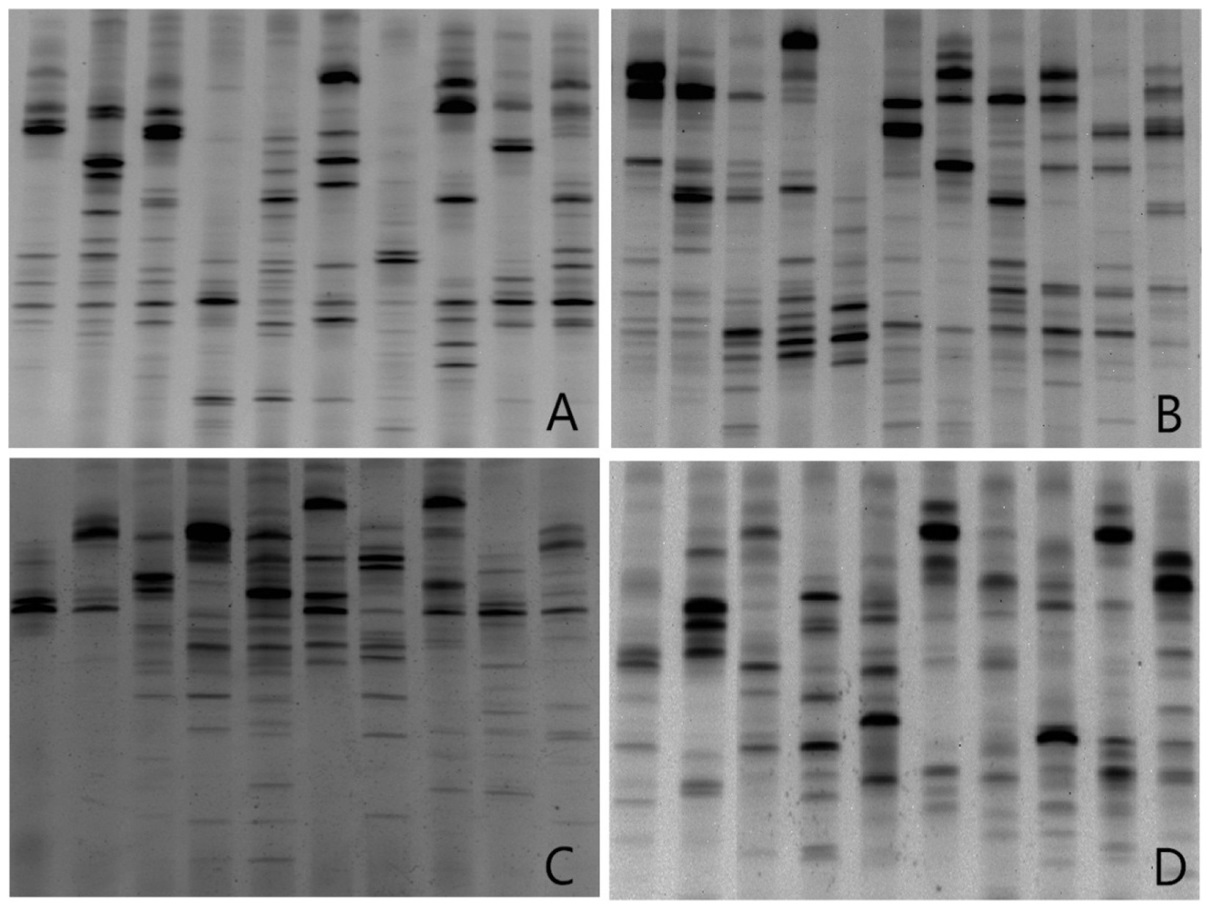
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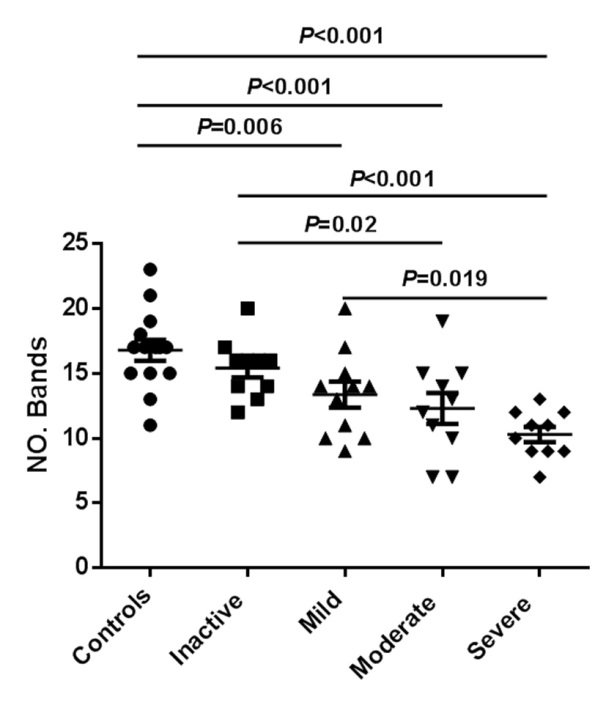
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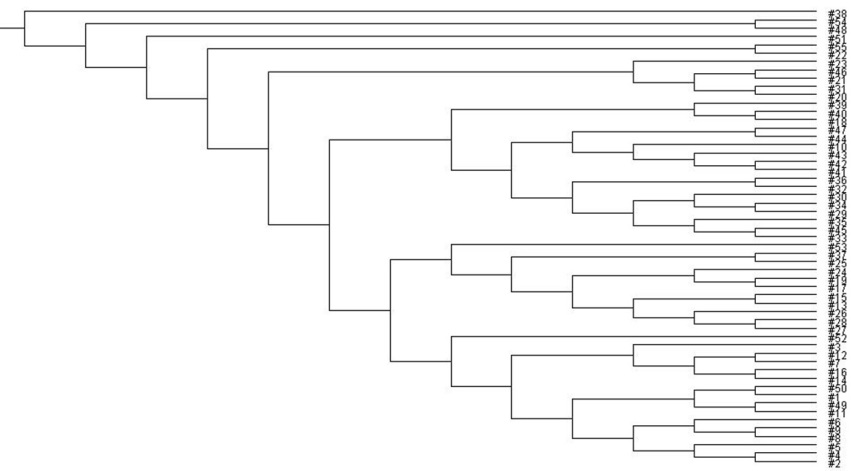
**Figure 1 Denaturing gradient gel electrophoresis profiles of fecal samples from healthy controls.** A: Denaturing gradient gel electrophoresis (DGGE) profiles; B: marked DGGE profiles. DGGE bands showed relative stability among different individuals.



**Figure 2 Denaturing gradient gel electrophoresis profiles showed microbial biodiversity in different ulcerative colitis groups.** A: Ulcerative colitis (UC) patients in remission; B: UC patients in mildly active stage; C: UC patients in moderately active stage; D: UC patients in severely active stage.



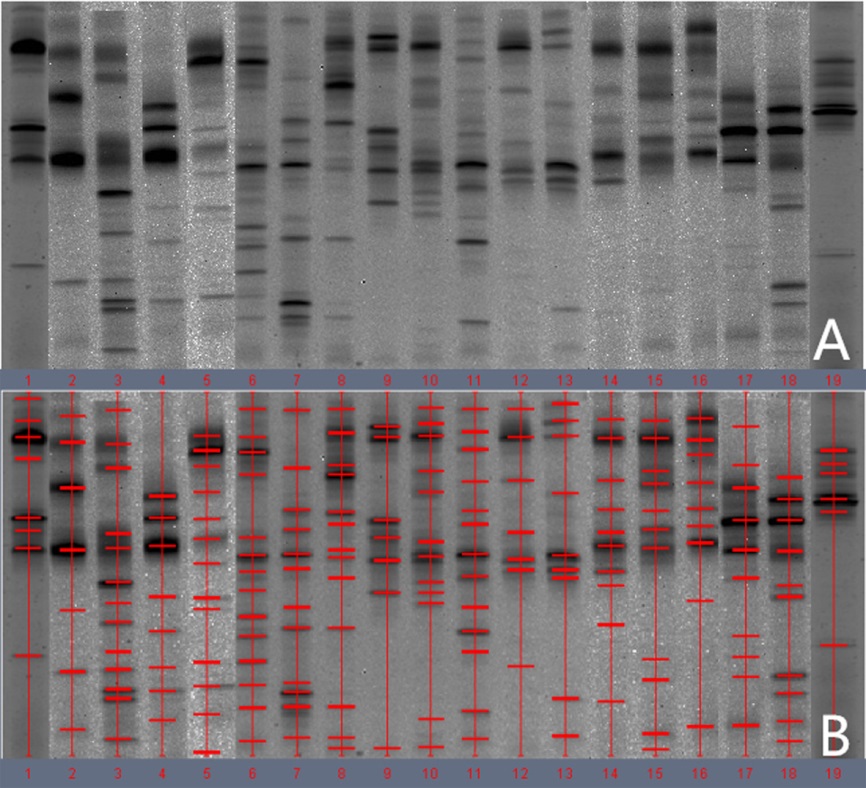
**Figure 3 Number of bands in denaturing gradient gel electrophoresis profiles of samples obtained from 41 ulcerative colitis patients.** The number of bands was reduced significantly from healthy controls to severe ulcerative colitis (UC).



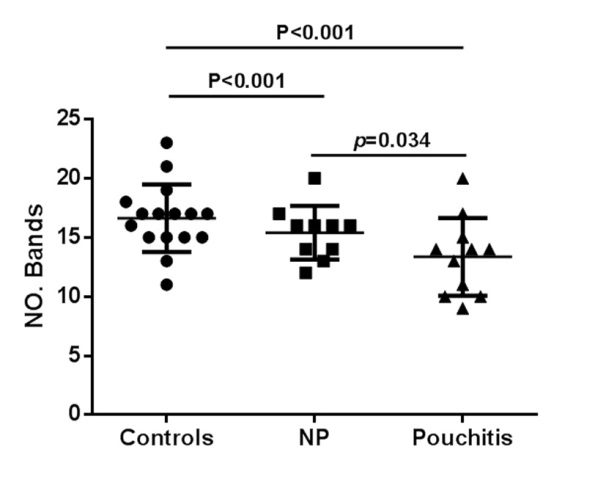
**Figure 4 UPGMA tree analysis of healthy people and ulcerative colitis patients in different stages.** 1-16: healthy people; 17-26: ulcerative colitis (UC) patients in remission; 27-37: UC patients in mildly active stage, 38-47: UC patients in moderately active stage; 48-57: UC patients in severely active stage. UPGMA tree analysis showed a significant difference among groups of healthy people and UC patients in different stages.



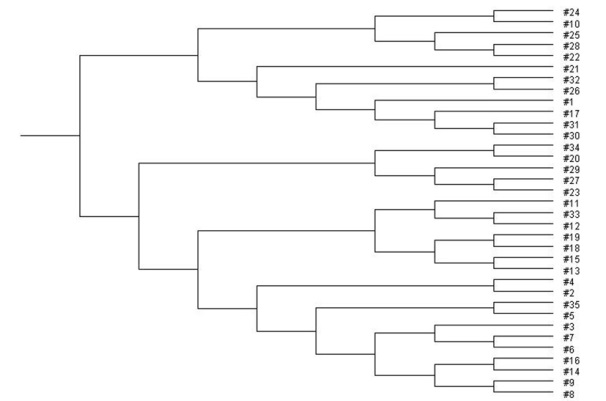
**Figure 5 Principal Component Analysis of denaturing gradient gel electrophoresis microbial profiles in fecal samples of healthy people and ulcerative colitis C patients in different stages.** Clustering of similar microbial profiles showed systematic differences among different groups.



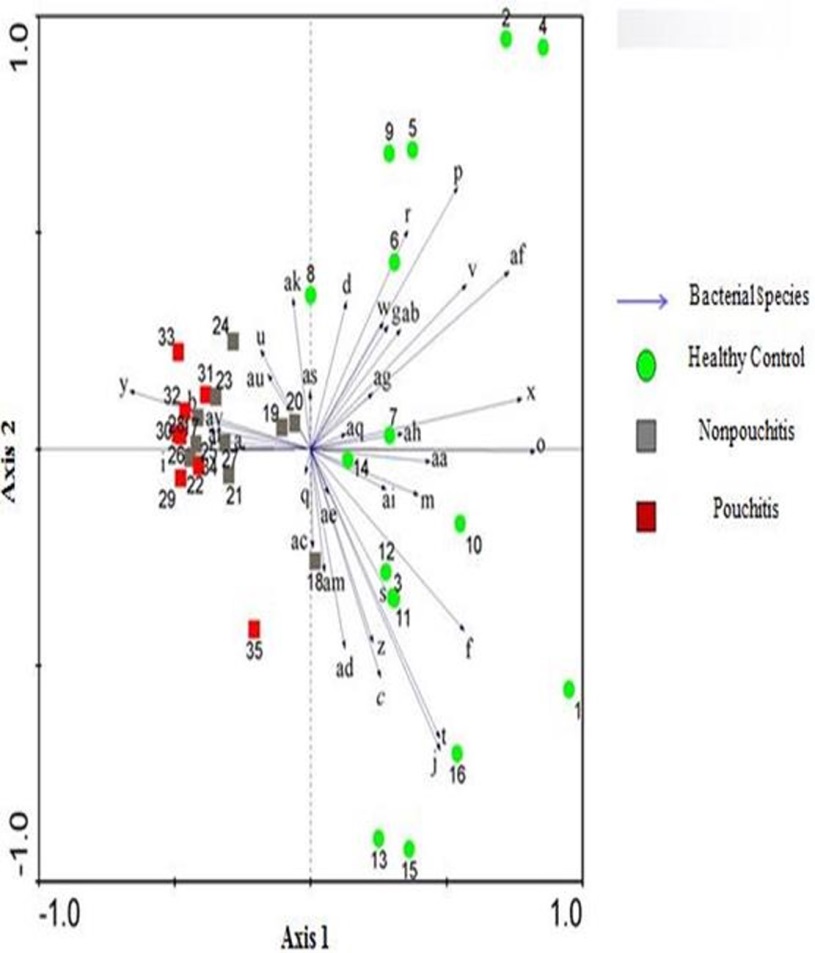
**Figure 6** **Denaturing gradient gel electrophoresis profiles of fecal samples from patients with pouchitis.** A: Denaturing gradient gel electrophoresis (DGGE) profiles; B: Marked DGGE profiles. DGGE bands revealed relative stability of microbiota in pouchitis group changing.



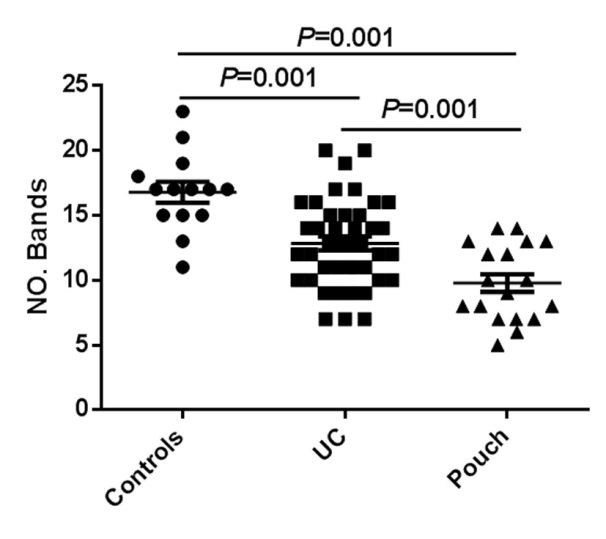
**Figure 7 Number of bands in denaturing gradient gel electrophoresis profiles of samples obtained from patients performed surgery.** The number of bands was reduced significantly in pouchitis compared with control group and no pouchitis group.



**Figure 8 UPGMA tree analysis of healthy people and postoperative patients.** 1-16: healthy people; 17-27: patients without pouchitis; 28-35: patients with pouchitis. UPGMA tree analysis showed a significant difference among three groups.



**Figure 9 Principal Component Analysis of denaturing gradient gel electrophoresis microbial profiles in fecal samples of healthy people and patients with pouch.** Clustering of similar microbial profiles showed significant differences among three groups.



**Figure 10 Number of bands in denaturing gradient gel electrophoresis profiles of samples obtained from all subjects.** The number of bands was reduced significantly in pouch group compared with control group and ulcerative colitis (UC) group.

**Table 1 Demographic and clinical characteristics of patients**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy controls** | **UC (*n* = 41)** | | | |  | **Pouch (*n* = 19)** | |
| Remission | Mild | Moderate | Severe | Pouchitis | | NP |
| Number of patients | 16 | 10 | 11 | 10 | 10 | 8 | | 11 |
| Sex, *n,* M/F | 9/7 | 5/5 | 7/4 | 5/5 | 6/4 | 5/3 | | 5/6 |
| Age (yr) | 46.2 ± 10.5 | 41.8 ± 9.2 | 43.9 ± 10.5 | 46 ± 8.2 | 40.8 ± 11.3 | 44.9 ± 15.6 | | 47.8 ± 13.2 |
| UC duration (yr) | NA | 5 ± 1.6 | 7 ± 0.9 | 5.2 ± 1.7 | 4 ± 2.7 | NA | | NA |
| Pouch duration (yr) | NA | NA | NA | NA | NA | 2.8 ± 1.5 | | 3.9 ± 2.2 |
| BMI | 24.5 ± 2.3 | 25.2 ± 1.6 | 24.8 ± 3.5 | 23.9 ± 2.7 | 24.4 ± 3.1 | 24.9 ± 2.2 | | 24.3 ± 3.5 |
| Mayo score | NA | ≤ 2 | 4.2 ± 0.7 | 8.5 ± 1.2 | 11.5 ± 0.3 | ≥ 7 | | < 7 |
| Age at colectomy | NA | NA | NA | NA | NA | 40.6 ± 12.9 | | 42.4 ± 9.22 |
| Standard medication (%) | NA | 0 (0) | 5 (46) | 8 (80) | 10 (100) | 8 (100) | | 3 (27) |
| Smoking (% at recruitment) | 10 (62.5) | 4 (40) | 3 (27.3) | 2 (20) | 3 (30) | 4 (50) | | 5 (45.5) |
| Previous number of episodes of pouchitis (%) | NA | NA | NA | NA | NA | 4 (50) | | 3 (27) |
| Number of patients with chronic pouchitis (%) | NA | NA | NA | NA | NA | 3 (37.5) | | 2 (18) |
| Secondary causes of pouchitis (%) | NA | NA | NA | NA | NA | 2 (25) | | 2 (19) |

UC: Ulcerative colitis; NA: Not available.

**Table 2 Dice analysis of normal control and ulcerative colitis subgroups**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Control** | **Remission** | **Mild** | **Moderate** | **Severe** |
| Control | (49.79 ± 11.24)%1 | (35.32 ± 14.86)% | (30.13 ± 11.23)% | (31.98 ± 16.48)% | (28.18 ± 14.99)% |
| Remission |  | (42.89 ± 18.29)%1 | (32.79 ± 13.68)% | (30.22 ± 15.28)% | (26.28 ± 13.94)% |
| Mild |  |  | (41.83 ± 16.38)%1 | (29.89 ± 13.10)% | (28.31 ± 18.39)% |
| Moderate |  |  |  | (43.45 ± 21.32)%1 | (28.88 ± 13.69)% |
| Severe |  |  |  |  | (37.12 ± 19.98)%1 |

1*P <* 0.05; result from similarity in the same group *vs* similarity in different groups.

**Table 3 Dice analysis of normal control and pouch subgroups**

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Control** | **Pouchitis** | **NP** |
| Control | (49.79 ± 11.24)%1 | (25.33 ± 11.13)% | (28.86 ± 14.23)% |
| Pouchitis |  | (35.43 ± 13.30)%1 | (20.87 ± 12.31)% |
| NP |  |  | (35.39 ± 10.80)%1 |

1*P <* 0.05; result from similarity in the same group *vs* similarity in different groups. NP: No pouchitis.

**Table 4 Bacterial diversity comparison**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Healthy controls** | **UC** | | | |  | **Pouch** | |
| Remission | Mild | Moderate | Severe | NP | | Pouchitis |
| Healthy controls | - | 0.298 | 0.0061 | 0.0011 | 0.0001 | 0.0001 | | 0.0001 |
| Remission UC | 0.298 | - | 0.113 | 0.0201 | 0.0001 | 0.0141 | | 0.0031 |
| Mild UC | 0.0061 | 0.113 | - | 0.404 | 0.0191 | 0.0071 | | 0.125 |
| Moderate UC | 0.0011 | 0.0201 | 0.404 | - | 0.128 | 0.0091 | | 0.448 |
| Severe UC | 0.0001 | 0.0001 | 0.0191 | 0.128 | - | 0.0191 | | 0.496 |
| NP: PDAI < 7 | 0.0001 | 0.0141 | 0.0071 | 0.0091 | 0.0191 | - | | 0.0341 |
| Pouchitis: PDAI ≥ 7 | 0.0001 | 0.0031 | 0.0051 | 0.448 | 0.496 | 0.0341 | | - |

1*P <* 0.05. NP: No pouchitis; UC: Ulcerative colitis; PDAI: Pouchitis Disease Activity Index.