Assessment of Gut Microbiome, their Relationship to Severity and Response to Treatment in Ulcerative Colitis patients.

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Abstract

Background: intestinal microflora plays an important role in health and disease and it plays a great role in ulcerative colitis patients.

Patients and methods: Acase control followed by cohort study carried out in Internal Medicine Department, and Immunology Departments, Faculty of Medicine, Zagazig university hospitals**.** All subjects submitted to full history taking, clinical examination, colonscopy and laboratory Investigations. Assessment of fecal microbes was done.

Results:. There is significant increase in lactobacilli or vionella after treatment in those with remission while there is non-significant increase in relapse**.** There is statistically non-significant relation between outcome and bacteroid before treatment however, there is significant difference between them after treatment (higher in remission). Within each group, there is significant decrease in bacteroid after treatment**.** There is statistically significant relation between severity after treatment and fecal, bacteroid and lactobacilli level after treatment.

Conclusion: fecal microbiome are highly specialized structure, diagnosis and monitor ulcerative colitis can be done based on fecal microbiome

Keywords: Ulcerative Colitis; Severity; Outcome.

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INTRODUCTION

Ulcerative colitis (UC) is a sort of chronic recurrent disorder with the characteristics of intestinal mucosa inflammation and ulceration. This disease causes significant morbidity worldwide, with morbidity and prevalence increasing over time **(Ungaro et al.,2017)**.

The microenvironment of the gut forms a good microbiome habitat, which has been demonstrated to affect many physiological conditions **(Faith et al., 2013)**. Since intestinal microbiome is considered as an important organ of the human body in recent times, an increasing number of studies have linked this microenvironment to gastrointestinal diseases. Because the composition of the intestinal microbiome is stable over a period of time, many studies inferred the gut microbiome as a potential predictor of health status and a target for therapeutic interventions **(Turnbaugh et al.,2009).**

Evaluation role of gut microbiome in disease course of new onset treatment naïve UC patients, they were monitored for one year and microbial taxonomic composition was analyzed from fecal sample, depletion of core gut microbiome and expansion of bacteria typical for oral cavity were associated with base line disease activity (**Schrimer et al.,2018)**

Potentially gut microbiota can drive pathogenicity via two mechanisms, expansion of proinflammatory species or restriction in the protective species **(Varela et al.,2013)**

Acondition of alteration of gut microbiome is called (dysbiosis),which in turn lead to alteration of immune system homeostasis, this condition is frequent in inflammatory bowel disease **(Davide et al.,2019).**

Therefore, this study aimed to assess the presence and severity of alteration of gut microbiome and occurrence UC patients. Also, to evaluate the changes in gut microbiome composition during the disease course.

PATIENTS AND METHODS

cohort study carried out in the Gastroenterology and Hepatology Unit, in Internal Medicine Department, and Medical Microbiology and Immunology Departments, Faculty of Medicine, Zagazig university hospitals**.**

Patients were informed for different diagnostic and treatment options and a written informed consent was signed. The study was approved from ethical committee of the hospital and Zagazig University Institutional Review Board (IRB)

Inclusion criteria:

Adult patients>18 years of both sex ,they were divided into 2 groups:

Group(1): Adult patients with ulcerative colitis diagnosed by clinical examination, colonoscopy, pathological examination. New-onset, treatment-naïve patients. Those patient classified according to severity based on colonscopic finding divvied into subgroups based on Mayo clinic sub score:

score 0: normal or inactive disease,

score1:mild (erythema, decrease vascular pattern, mild friability),

score2: moderate (marked erythema, absent vascular pattern),

score3:sever (ulcer with spontaneous bleeding).

(Subgroup A):mild to moderate inflammation, **(Subgroup B)**:sever inflammation. They received treatment based on guide lines (steroid, 5ASA, azathioprine) followed up after 6m.

Remission of UC in practice defined as stool frequency>3/day with no bleeding or urgency. Relapse is defined as aflare of symptoms in patient with established UC who is in clinical remission whether spontaneously or after medical treatment and according to ECCO consensus rectal bleeding is essential component **(Dignass et al., 2012)**.

Exclusion criteria:

Use of antibiotics or corticosteroids in the previous 3 months. Use of non-steroidal anti-inflammatory drugs (NSAIDs) in the previous 3 months. Reported recent diagnosis (less than 3 months) of bacterial or parasitic infections of the gastrointestinal tract. Pregnancy and breast feeding.

Operational design:

All subjects submitted to full history taking, clinical examination, colonscopy and laboratory Investigations including complete blood count, liver function tests, blood urea and serum creatinine, PT , PTT ,INR. Serum electrolytes: Na, K, Ca, Po4 , Mg.

Assessment of fecal microbiome:

Collection of fecal samples: Approximately 10 g of fresh stool samples (selected from different parts of the stool) were obtained from each subject. Fecal samples were collected again from patients 6 months later. All samples were preserved at -20o C till the time of use.

-Microbial Genomic DNA Extraction: genomic DNA was extracted from fecal samples using a QIAGEN stool kit (QIAGEN, Hilden, Germany) from 200mg feces following the manufacturer's instructions.

-Amplification by conventional PCR to check primer specificity. A conventional PCR was performed using the recommended thermal cycling conditions in Bio-Rad PCR machine (Bio-Rad, USA). Primers were purchased from (operon, Invitrogen). PCR reactions consisted of 35 cycles, with an initial DNA denaturation at 95°C (30 s), followed by gradient annealing temperature (30 s) and elongation at 72°C (45 s). The procedure was completed with a final elongation step at 72°C (10 min). Amplified PCR products were identified using agarose gel electrophoresis.

Quantitative Real-Time PCR.

Quantification of gene copies of Bacteroides, Lactobacilli, , Veiollena, and Hemophilus groups was carried out for each sample using ROCHE LightCycler® 480 instrument (Sydney, Australia). Each PCR was carried out in a final volume of 10 µl, including template DNA, primers, and SYBR® Green PCR master mixture. Thermal cycling conditions started with reaction cycle at 95°C for 30 s followed by 40 cycles of initial denaturation at 95°C for 5 s and 20 s of annealing at 60°C.

Statistical analysis:

Data collected and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD. Differences between quantitative independent multiple by Z Mann Whitney test, WX Wilcoxon signed rank test, ANOVA or Kruskal Wallis,. P value was set at <0.05 for significant results &<0.001 for high significant result.

RESULTS

There is statistically significant difference between the studied case and control groups regarding F. prausnitzii, lactobacilli, Bacteroides, and Veillonella. F. prausnitzii and lactobacilli were significantly lower in UC patients ($p<0.001$) while both Bacteroides and Veillonella were significantly higher ($p<0.001$). Hemophilus was detected in small amount in UC patients, however it was not detected in the control group.

There is statistically significant increase in mean values of hemoglobin, serum albumin, and mean platelet volume with a significant decrease in mean value of white blood cell count, N/L ratio, platelet count, serum calprotectin and ESR 6 months after treatment in the UC group.

There is statistically significant increase in F. prausnitzii and Lactobacilli 6 months after treatment, with a statistically significant decrease in Bacteroides, Veillonella and Hemophilus 6 months after treatment .Comparing the microbial content in UC group after treatment with the control group showed a statistically significant difference regarding F. prausnitzii, Bacteroides, and Veionella. Though F. prausnitzii, Lactobacilli showed a statistically significant increase from baseline after treatment in the case group, yet, they are still lower when compared to the control group ($P<0.001$, 0.069 respectively). Both Bacteroides and Veionella were still higher when compared to the control group, despite the significant decrease from baseline mediated by UC treatment.

Clinical, endoscopic remission was achieved in 17/24 patients (70.8%) 6.2±1.5 months after therapy. Females represented 29.4% and 71.4% within those with remission or relapse respectively.

There is statistically significant relation between outcome and F. praustinizii being higher in remission. It showed a significant increase after treatment in those with remission.A significant increase in lactobacilli was noted after treatment in those with remission in contrary to patients with relapse. Bacteroides showed a significant decrease after treatment in subgroups with remission or relapse, however, it remained significantly higher after treatment in patients with relapse. Veillonella was significantly decreased after treatment in those with remission. However Hemophilus showed a higher content in patients with remission, with a significant decrease in patients with relapse.

There is statistically significant relation between site of affection and gut microbiome level; F. praustinizii and lactobacilli levels were inversely proportional with the extent of disease, being significantly more prevalent in proctosigmoid UC followed by left sided colitis and pancolitis, after treatment, they were significantly increased in pancolitis subgroup.

Bacteroides, Veillonella and Hemophilus were significantly higher in pancolitis, followed by left sided and proctosigmoid subgroups denoting that its level is directly proportional to the extent of the disease. Treatment significantly caused a decrease in their levels in pancolitis subgroup.

Table (1) Relation between outcome and demographic data

Parameter	Response		Test	
	Remission	**Relapse**	**t/ χ^2**	**p**
	Mean ± SD	**Mean ± SD**		
Age	39.12 ± 12.79	37.71 ± 16.97	0.222	0.836
Gender:	N=17	N=7		
Female	5 (29.4%)	5 (71.4%)	Fisher	0.085
Male	12 (70.8%)	2 (28.6%)		

χ^2 Chi square test /t Independent sample t test

Table (2) Comparison between the studied groups regarding gut microbes (PCR) before treatment

Parameter	Group		Test	
	Case group before treatment	Control group	t	P
	Mean ± SD	Mean ± SD		
Lactobacilli	5.86 ± 0.76	6.74 + 0.69	-3.776	<0.001**
Bacteroides	12.01 ± 2.25	9.84 ± 0.74	4.503	<0.001**
	Median (range)	**Median (range)**	**Z**	**P**
Veionella	1.86 (0.83 – 4.02)	1.0 (0.2 – 3.5)	-4.031	<0.001**

Z Mann Whitney, test t independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

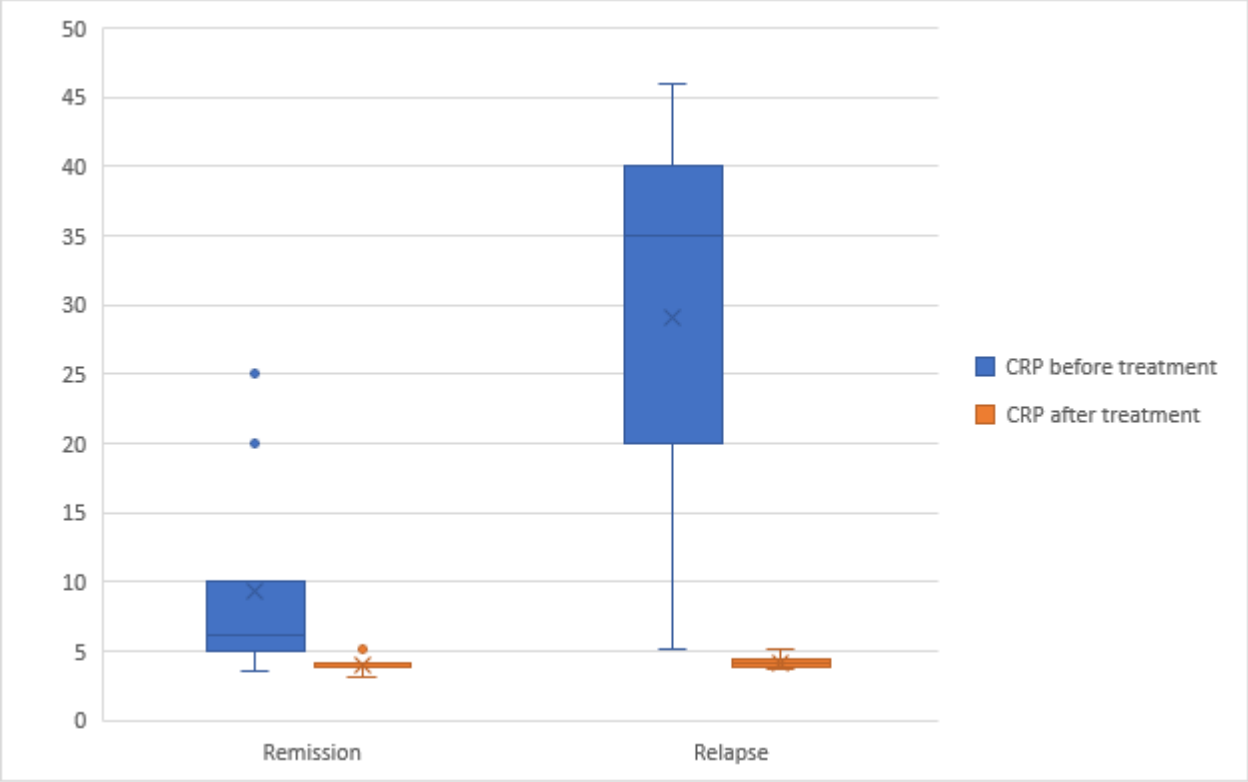

Figure (1) Boxplot showing relation between outcome and CRP before and after treatment among the studied patients

Table (3) Comparison between the studied groups regarding gut microbes (PCR):

Gut microbes	Response		Test	
	Remission	Relapse	t	p
	Mean ± SD	Median (range)		
Lactobacilli:				
Before	6.04 ± 0.51	5.42 ± 1.09	1.446	0.095
After	6.56 ± 0.55	6.03 ± 0.88	1.487	0.088

P (Wx)	0.002*	0.111		
Bacteroid :				
Before	11.61 ± 2.18	12.99 ± 2.25	-1.392	0.089
After	10.63 ± 1.32	12.62 ± 2.18	-2.259	0.027*
P (Wx)	<0.001**	0.004*		
Vionella:				
Before	1.49 (0.83 – 4.02)	2.03 (0.95 – 2.86)	-0.635	0.525
After	1.42 (0.79 – 3.92)	1.98 (1 – 2.76)	-0.572	0.567
P (Wx)	<0.001**	0.105		
Hemop:				
Before	1.42 (0.32 – 4.06)	3.22 (1.12 – 3.98)	-0.859	0.391
After	1.51 (0.25 – 2.73)	1.43 (1.01 – 3.72)	-0.672	0.502
P (Wx)	0.009*	0.018*		

Z Mann Whitney test WX Wilcoxon signed rank test **p≤0.001 is statistically highly significant

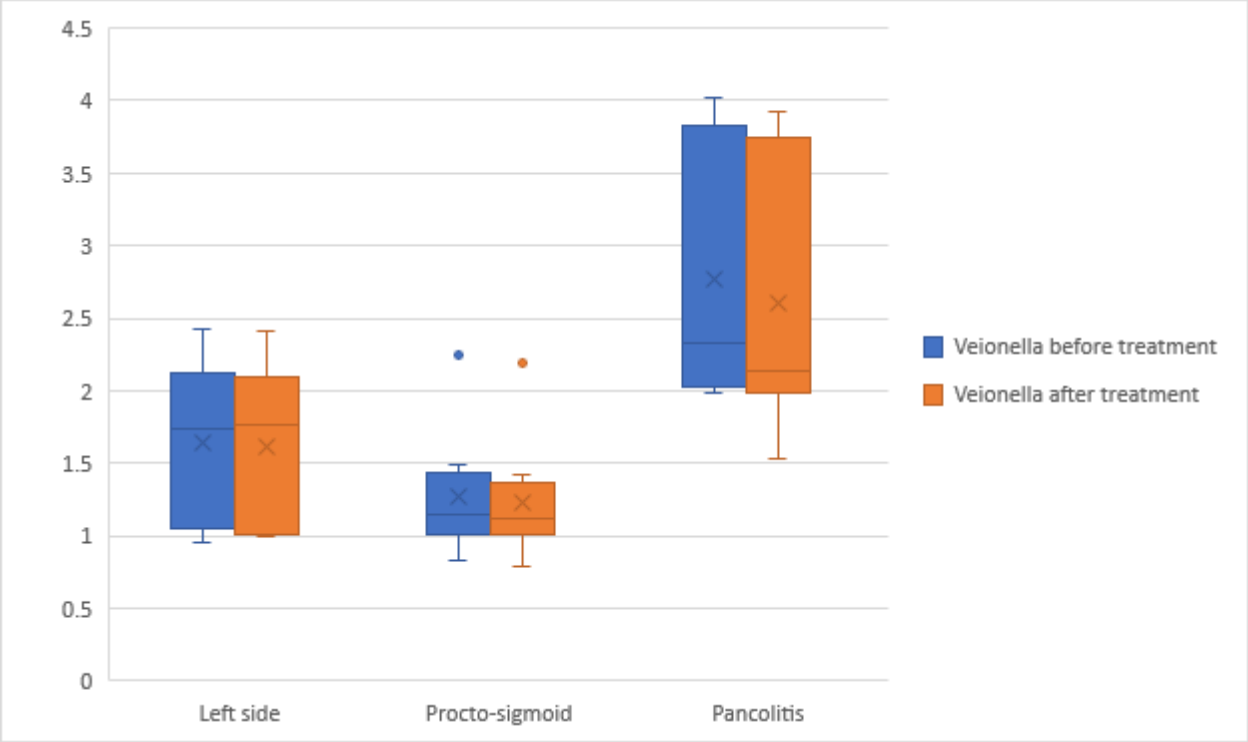

Figure (3) Multiple line chart showing Vionella before after treatment according to site of lesion

Table (4) Relation between severity and gut microbes before treatment among the studied patients:

Gut microbes	Site			Test	
	Mild (n=10)	Moderate (n=6)	Severe (n=8)	F	p
	Mean ± SD	Mean ± SD	Mean ± SD		
Lactobacilli	6.45 ± 0.31	5.8 ± 0.49	5.17 ± 0.73	13.458	**<0.001****
LSD	**P_1 0.02***	**P_2 0.035***	**P_3 <0.001****		
Bacteroid	10.07 ± 0.35	11.5 ± 0.35	14.83 ± 0.4	81.836	**<0.001****

LSD	**P_1 0.002***	**P_2 <0.001****	**P_3 <0.001****		
	Median (range)	**Median (range)**	**Median (range)**	**KW**	**p**
Veoniella	1.06 (0.95 – 2.25)	1.87 (0.84 – 2.43)	2.33 (1.99 – 4.02)	12.52	**0.002***
Pairwise	P_1 0.421	P_2 0.269	**P_3 0.001****		

P1 difference between mild and moderate p2 difference between moderate and severe p3 difference between mild and severe LSD Fisher least significant difference KW Kruskal Wallis test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant F One way ANOVA test

Table (5) Relation between severity and gut microbes after treatment among the studied patients:

Gut microbes	Site			Test	
	Mild (n=10)	**Moderate (n=6)**	**Severe (n=8)**	**F**	**p**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Lactobacilli	6.6 ± 0.55	6.24 ± 0.44	5.88 ± 1.16	2.115	0.146
Bacteroid	10.15 ± 0.37	12.32 ± 1.65	13.78 ± 2.1	19.29	**<0.001****
LSD	**P_1 <0.001****	**P_2** 0.068	**P_3 <0.001****		
	Median (range)	**Median (range)**	**Median (range)**	**KW**	**p**
Veoniella	1.23 (0.79 – 2.19)	2.41 (1.53 – 3.92)	2.04 (1.21 – 2.76)	6.64	**0.036***
Pairwise	P_1 0.**045***	P_2 >0.999	**P_3** 0.495		

P1 difference between mild and moderate p2 difference between moderate and severe p3 difference between mild and severe LSD Fisher least significant difference KW Kruskal Wallis test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant F One way ANOVA test

DISCUSSION

Ulcerative cholitis generally considered to arise from interaction between host genetics,environmental factors and desregulated immune response**. .(Sartor RB et al.,2008).** Alteration in intestinal microbiota composition consider to play central role in the pathogenssis of ulcerative cholitis**.(Sartor RB et al.,2008).** Numerous studies corroborated evidence of intestinal dysbiosis in ulcerative cholitis patients compared to healthy control but few studies investigate intestinal microbiome in relation to disease activity **(Baumgart DC et al.,2017).**

This study was designed to quantified Bacteroid,Lactobacillus,fecalbacterium ,Hemophilus and veionella and determine the difference between healthy control and recent diagnosed ulcerative cholitis patients and also evaluate their change in relation to disease severity by real time PCR.

Our study found significant change in gut microbiome between healthy and ulcerative cholitis patients and there is significant change in gut microbiome during disease course,between healthy and diseased there is significant decrease in Lactobacillus and increase in Bacteroidis ulcerative cholitis patients, Also there is no Veionella in healthy control and they significant appear in ulcerative cholitis patient.

The result of the study was the same as study done at university of Maastrich in Netherlands which was done to asses fecal bacterium in UC patients during remission and subsequent activity in which fecal sample collected from 10 ulcerative cholitis patients during remission and subsequent exacerbation ,microbial composition was assessed by 16srRNA,it

shows significant decrease in fecal bacterium during disease activity and increase in remission**(Ermann J et al.,2014).**

Also our result is similar to astudy done by Harry sokol etal in france in which bacterial composition in fecal sample was assessed by using 16sRNA in 20 UC patients and compare it with 13 healthy control at one point of time it found significant decrease in fecalbacterium ,increase in Bacteroid and decrease in Lactobacillus in UC patients**(Harbord M et al.,2018).**

Also it was similar to study done by Alexander Swidsinki etal in 2018 in Duke university center in NorthCalorina on 20 UC pt and compare them by 20 healthy control using 16RNA on mucosal biobsies taken during colonscopy they found that mucosal bacteria found at higher concentration in UC patients compared with healthy control and domination of Bacteroid in UC pt **(Angelberger S et al.,2013).**

A study done by **M.Alam etal** at university of Warwick UK in 2020 in which fecal sample collected from 30 UC pt and 15 healthy control using 16rRNA found significant abundant of bacteroid (Mohamed Alam etal.,2020).

F.J Rayan etal conducted astudy on 80 UC patients and 31 healthy control in Ireland using FISH technique on colonic mucosa and they follow UC patients for one year they found significant change between Healthy control andUC patients in which there is decrease in fecalbacterium and increase in Bacteroids but unlike us they don't found change in microbiome according to inflammatory status in UC patients **(Ramos etal.,2019).**

Unlike our study Lena ohman etal conducted astudy in university of Northcalorina on 40 UC patients in which fecal sample obtained from these patient when they were in remission and another sample taken during subsequent activities which found no significant change in probe signal intensity of the major 4 phyla **(O conner etal.,2018).**

Similar to our study Roding Bibilo etal conduct astudy on 20 recently diagnosed UC patients and 12 healthy control in Canda using 16rRNA on biobsies sample ,they found that UC patients has more Bacteroids and less fecalbacterium**(Baumgart DC et al.,2017).**

Un like our study Tom Vanhoutte etal conduct astudy in Belgium in 2014 on 50 UC patients and 30 healthy controls using 16rRNA on fecal sample which found decrease in Fecalbacterium and lactobacillus and increase in Bacteroids in relation to healthy control and they follow up those patient but they didn't found significant change in microbial composition in relation to remission and activity**(Turner D et al** .,2017). **Annese V** conducted astudy in Berlin in 2018 on 80 UC patients and 32 healthy patients using FISH technique on stool sample found depletion of Fecalbacterium while in healthy individual Fecalbacterium still present and increase Bacteroid in UC patients

Un like our result **Shouval DS** conducted astudy in Ireland in 2016 who conducted study on 16 UC patiets and 11 healthy individual using 16rRNA found domination of Bacteriod in fecal sample with temporal stability in fecal microbiome with no change related to disease activity

Similar to our study **Moran C** conducted a study in USA in 2018 using 16rRNA on fecal sample of 20 UC patients and 18 healthy control found expansion of oral microbiome like Hemophilus and veionella in fecal sample of UC patients and it correlate with disease severity .

Kaplan GG et al conducted astudy in 2018 in Ireland analyze salivary microbiome and correlate it with fecal sample in 35 UC patients compared with 20 healthy control using

16srRNA found depletion in oral microbiome in saliva and expansion in fecal sample it though that oral gut transition lead to immunogenic stimulation of the gut.

Assesment of Ulcerative cholitis depends on clinical presentation together with radiological investigation ,endoscopic and Histopathological examination,Endoscopy is gold standard but may not be applicable due to possible complication in active Ulcerative cholitis so the objective to search for alternative to evaluate those patients and achieve the aim of treatment which is endoscopic and clinical remission **(Okba etal.,2019).**

CONCLUSION

There is a difference in gut microbiome between healthy control and recently diagnosed ulcerative colitis patients,UC pt has lower levels of fecalbacterium,and higher level of bacteroids and veionella.

Oral microbiome like Veionella are not found in healthy control but appear in Ulcerative colitis patient stool Fecal calprotectin assay can be used to detect subclinical activity.

No Conflict of interest.

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