Study Protocol and Statistical Analysis Plan

Short-term Effects of Bowel Preparation on Gut Microbiome in Patients Undergoing Endoscopic Colon Polypectomy

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I. Background of the study

Good and adequate bowel preparation is essential for performing colonoscopy, especially for patients requiring endoscopic treatment, and the role of bowel cleansing agents (i.e., laxatives) and their safety for patients have been extensively studied [1]. A variety of laxatives are used in clinical practice, the most widely used being electrolyte-supplemented polyethylene glycol (PEG) [2], a PEG solution that rapidly passes through the gastrointestinal tract causing severe osmotic diarrhea, altering the contents of the intestinal lumen, including the microbiota [3]. In addition, other types of laxatives have been developed, such as compounded sodium picosulfate granules, which stimulate peristalsis of the intestinal mucosa and act as an osmotic laxative, increasing the water content of the intestinal lumen, and are taken with a slightly better taste than PEG.

When a large amount of laxative passes through the gut, many microorganisms are removed from the gut. This results in significant changes in the composition of the gut microbiota during the intestinal cleansing process. A number of studies have been reported on the changes in the gut microbiome produced after gut cleansing in healthy populations [4-8] to assess whether gut cleansing directly leads to dysbiosis, whether the flora is restored, and to explore the impact of this process on health and disease. However, there is no consensus on how gut cleansing affects the gut microbiome. Some studies have found an increase in the relative abundance of Bacteroidetes and Ascomycetes and a decrease in the relative abundance of Thick-walled Bacteroidetes following gut cleansing [4, 9], however, others have found that gut cleansing does not have a significant effect on the microbiome [3, 7].

Colon polyps are a common gastrointestinal tumor, and most of these lesions are histologically adenomatous, with a risk of becoming cancerous [10], showing an "adenoma-adenocarcinoma" progression, so complete resection is essential. In most patients, polyps can be completely removed at an early stage by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) to avoid progression to colon cancer [11].

There are still no studies reporting the effects of laxatives on the gut microbiome of patients with colon polyps, and in addition, most of the current studies, focusing on the effects on the gut microbiome after bowel cleansing with PEG [4, 7, 8, 12], have not explored whether other types of laxatives have different effects on the human gut microbiome. In our study, we plan to focus on different types of laxatives and explore whether they have different effects on the gut microbiome of patients with intestinal polyps after bowel cleansing, as well as to analyze whether the diameter of the colon polyps, pathological findings, and other baseline information also lead to different changes in the composition, evolution, and recovery of the gut microbiome of the patients, and to further explore the effects of microbiome changes on the patients' disease, and health.

II.Purpose of the study:

To investigate the role of different types of laxatives (compounded polyethylene glycol electrolyte dispersions and compounded sodium pico-sulfate) on the composition, evolution and recovery of the intestinal microbiome of patients with colonic polyps undergoing bowel preparation.

III.Overview of the study:

1.Study Steps:

Patients requiring e-colonoscopy and treatment for colon polyps were recruited and randomized into a polyethylene glycol group and a sodium picosulfate group. Patients in the two groups were prepared for bowel preparation with cotrimoxazole polyethylene glycol electrolyte dispersions and cotrimoxazole sodium picosulfate, respectively, while basic information about the patients was collected. Patients were left with feces before and after oral laxatives, and feces were left again 7 and 14 days after the end of intestinal cleansing, and intestinal microbiome sequencing was carried out to observe the changes in the intestinal microbiome of patients. The diameter of colonic polyps was recorded during the patients' operation, and the pathological results of colonic polyps were followed up after the operation.

2.Study population:

Patients who need to undergo electronic colonoscopy and treatment due to colon polyps.

3.Inclusion criteria:

1)All colon polyps diagnosed by electronic colonoscopy.

2)Age above 18 years old.

3)Patients sign an informed consent form, agree to cooperate with the study of this project, and collect fecal specimens on time to receive follow-up visits.

4. Exclusion criteria:

1)Patients who received antibiotics, PPIs 2 weeks before the study.

2)Patients who consumed probiotics or herbs 2 weeks prior to the study.

3)Had a colonoscopy or used diarrhea-inducing drugs or gastrointestinal stimulants 1 week prior to the study.

4)Gastrointestinal surgery and gastrointestinal endoscopic procedures in the 1 month prior to the study.

5)Bacterial or parasitic intestinal infections in the 1 month prior to the study.

6)Patients on long-term low-calorie diets, vegan diets, gluten-free diets and other "special" diets.

7)Pregnant/nursing patients.

8)Patients with a history of hypersensitivity to relevant medications.

9)Patients with contraindications to sodium picosulfate: renal insufficiency, renal transplant recipients, congestive heart failure, symptomatic ischemic heart disease within the last 6 months, cirrhosis of the liver, patients on hemodialysis or peritoneal dialysis, patients taking certain medications - renin-angiotensin blockers, diuretics, non-steroidal anti-inflammatory drugs (NSAIDs), and patients taking medications known to trigger the syndrome of inappropriate secretion of antidiuretic hormone. patients taking medications known to induce the syndrome of inappropriate antidiuretic hormone secretion (tricyclic antidepressants, selective 5-hydroxytryptamine reuptake inhibitors, multiple antipsychotics, and carbamazepine).

10)Patients with contraindications to colonoscopy, such as severe hypertension, anemia, coronary artery disease, cardiopulmonary insufficiency, etc..

11)Patients with contraindications to drug-induced diarrhea, such as intestinal obstruction, electrolyte disorders, or severe renal insufficiency.

12)Any other reason the investigator considers inappropriate for enrollment.

IV Statistical analysis:

Calculation of multiple Alpha diversity indices (richness, Chao1, Shannon, etc.) based on the (Z)OTU abundance table was performed using the USEARCH alpha_div subcommand (version 11.2.64). USEARCH beta_div was used to calculate the beta diversity distance matrices between samples, including weighted_unifrac, unweighted_unifrac and bray_curtis. Based on the three beta diversity distance matrices, PCoA analysis was performed and plotted using the R software, and based on the homogenization of OTUs. abundance table, PCA analysis was performed and plotted at different taxonomic levels using the gmodels package of R software, NMDS analysis was performed and plotted based on the three beta diversity distance matrices using the NMDS package of R software, and the three beta diversity distance matrices computed based on the homogenized OTU abundance table were analyzed and plotted using the Vegan package of R software (Version 2.4.2). Anosim analysis and mapping. Differential species composition was identified using DESeq2 (Version 1.26.0). Functional information in the samples was mined based on 16S sequences, mainly using the KEGG database of known bacteria and their functional annotation information for prediction. The investigators used the BLAST method to compare the (Z)OUT representative sequences with the SILVA_123_SSURef_Nr99 database to complete the sequence classification of the OTUs, and then used the Tax4Fun (version 0.3.1) package for R to obtain detailed abundance information of the KOs, and used the high level KEGG annotations of the metabolic pathways, enzymes, reactions, etc., which were associated with the KOs. information, and finally statistically analyzed and presented.

The routine data were processed using SPSS 25.0 statistical software, and the measurement information was tested by Kolmogorov-Smirnov test for conformity to normal distribution; the measurement information conforming to normal distribution was expressed as mean ± standard deviation, and the comparison between groups was performed by independent samples t-test; the measurement information not conforming to normal distribution was expressed as median and 25%-75% quartiles, and the comparison between groups was performed by Mann-Whitney U test; count data were described as the number of cases and percentages, and comparisons between groups were made using the □2 test, with successive corrected chi-square values when the theoretical frequency of any cell was $1 < E < 5$ and Fisher's exact test when the theoretical frequency of any cell was $\leqslant 1$; hierarchical data were used with the Mann-Whitney U test;

V. Research-related ethics

1. Ethics Committee Review

The protocol and written informed consent and information directly related to the subjects must be submitted to the Ethics Committee, and the study can be formally conducted only after obtaining written approval from the Ethics Committee. The investigator must submit an annual report of the study to the Ethics Committee at least once a year. The investigator must notify the Ethics Committee in writing upon termination and/or completion of the study; the investigator must report to the Ethics Committee in a timely manner all changes occurring in the conduct of the study (e.g., revisions to the protocol and/or informed consent form) and must not implement these changes without first obtaining the approval of the Ethics Committee unless the changes are made in order to eliminate a clear and immediate risk to

the subjects. The Ethics Committee will be notified when this occurs.

2. Informed Consent

The investigator must provide the subject or his/her legal representative with a readily understandable and Ethics Committee-approved informed consent form and allow the subject or his/her legal representative sufficient time to consider the study, and the subject will not be enrolled until a signed written informed consent has been obtained from the subject. Subjects will be provided with all updated versions of the Informed Consent Form, as well as written information, for the duration of their participation. The informed consent form is retained for review as an important document of the clinical trial.

VI. Confidentiality Measures

The results of the research through this program may be published in medical journals, but the investigators will maintain the confidentiality of patients' information in accordance with the requirements of the law, and patients' personal information will not be disclosed unless required by relevant laws. If necessary, the government administration and the hospital ethics committee and its related personnel can access the patients' information according to the regulations.

VII. Expected progress and completion time of the project

This study is expected to be completed by September 30, 2023

VIII. References (up to 20)

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