Research Protocol

Clinical Experimental Study on the Impact of Bowel Preparation with PEG Laxatives on Gut Microbiota in Patients Undergoing Cholecystectomy

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1.Research Background

Colonoscopy is a necessary item for colorectal cancer screening. For high-risk populations, annual screening is the most effective method for early prevention. Currently, in colonoscopy, a large amount of polyethylene glycol (PEG2000-4000) is used as a laxative and lubricant. The extensive use of PEG can cause long-term and irreversible damage to gut microbiota, posing significant risks to health[1].

The gallbladder is a sac-like organ located below the liver, whose main functions include storing and concentrating bile, regulating biliary pressure, participating in the digestion and absorption of fats, and protecting the intestinal mucosa[2]. Due to the prevalence of gallbladder diseases such as gallstones and cholecystitis, cholecystectomy is a common surgical procedure worldwide[3]. Laparoscopic cholecystectomy (LC) has become the preferred surgical method for benign gallbladder diseases due to its advantages of minimal invasiveness and rapid recovery[4]. However, cholecystectomy may affect the digestive function of patients[5,6]. After surgery, bile acids are continuously discharged into the duodenum, which may lead to symptoms such as indigestion and steatorrhea[6,7], and even promote the development of colorectal cancer[8]. In addition, cholecystectomy may affect the composition of gut microbiota, thereby impacting host health[9].

This study will observe the impact of PEG on gut microbiota, analyze changes in the intestinal microecology before and after colonoscopy, design better bowel cleansing agents and intestinal repair probiotics, and further reduce the adverse effects of colonoscopy on patients who have undergone cholecystectomy. Additionally, it will explore the interaction between the gallbladder and gut microbiota and its significance.

2. Research Objectives

The study plans to recruit a group of individuals who have undergone cholecystectomy for colonoscopy: (1) Observe changes in gut microbiota before and after colonoscopy in cholecystectomy patients; (2) Analyze whether the recovery of gut microbiota changes before and after colonoscopy in cholecystectomy patients differs from that in the normal population.

3. Study Design

This study adopts a parallel controlled observational clinical trial method to investigate the

short-term and long-term effects of PEG use on patients' gut microbiota, including tracking and analyzing the recovery and damage of gut microbiota. It will also compare the recovery and damage status of microbiota with those in individuals who have not undergone cholecystectomy, and design corresponding microecological repair probiotics based on this. Bioinformatics methods will be used for statistical analysis of sample data.

4. Selection of Subjects

1. Inclusion Criteria

Subjects will be mainly recruited who meet the following criteria: 1) Have undergone cholecystectomy; 2) Aged 18-75 years; 3) Planning to undergo colonoscopy; 4) In good health with no history of major organ diseases.

Control subjects will be recruited who meet the following criteria: 1) Aged 18-75 years; 2) Have not undergone cholecystectomy; 3) In good health with no history of major organ diseases.

2. Exclusion Criteria

Subjects will be excluded if they meet the following conditions: (1) Have contraindications for colonoscopy, such as cardiopulmonary insufficiency; (2) Unable to tolerate PEG laxatives; (3) Pregnant women; (4) Mental disorders. The control group consists of individuals undergoing colonoscopy who have not undergone cholecystectomy; (5) Have used antibiotics within half a year.

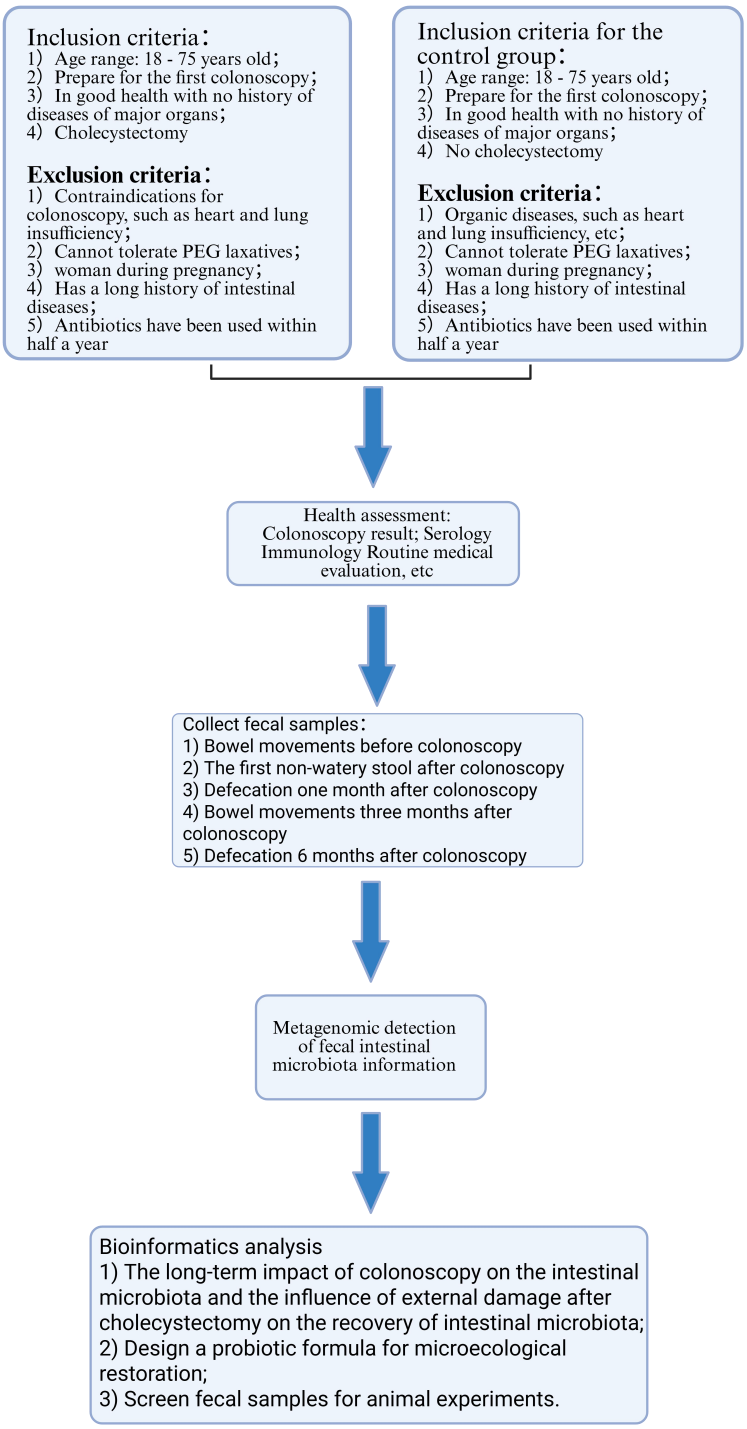
Control subjects will be excluded if they meet the following conditions: (1) Have organic diseases, such as cardiopulmonary insufficiency; (2) Pregnant women; (3) Mental disorders; (4) Have used antibiotics within half a year.

5. Sample Size Estimation

The number of participants included in this study is approximately 20.

6. Study Process

The technical route of this study is as follows:



7. Potential Risks and Preventive Measures

Potential discomforts in this study include psychological and sensory discomfort caused by fecal sample collection at home, such as nausea and vomiting. Each fecal sampling kit is equipped with a pair of gloves and two pieces of toilet paper.

8.Data Collection and Statistical Analysis

Patients' fecal samples will be sent to the company for metagenomic sequencing to obtain microbial metagenomic data from the fecal samples.

Raw files obtained from the DNBSEQ-T7 platform are converted into short reads (raw data) through base calling, and these short reads are recorded in FASTQ format, which contains sequence information and corresponding sequencing quality information.[30]

Sequence artifacts, including reads containing adapter contamination, low-quality nucleotides, and unidentifiable nucleotides (N), undoubtedly pose obstacles to subsequent reliable bioinformatics analysis. Therefore, quality control is a necessary step to ensure meaningful downstream analysis.

Fastp (version 0.23.1)[31]was used to perform basic statistics on the quality of raw reads.

The data processing steps are as follows:

(1) If one of the reads contains adapter contamination, discard the paired reads;

(2) If the number of ambiguous bases exceeds 10% in any read, discard the paired reads;

(3) If the proportion of low-quality bases (Phred quality < 5) exceeds 50% in any read, discard the paired reads.

After passing quality control, each sample requires a total of 0.2g DNA as input material for DNA library preparation. First, genomic DNA samples are ultrasonically sheared using a Covaris ultrasonic disruptor to meet the size requirement of 350bp. Then, end repair, A-tailing, addition of sequencing adapters, fragment screening, PCR amplification are performed, followed by purification of products using the AMPure XP system (Beverly, USA). Library quality is evaluated and quantified on the Agilent 5400 system (Agilent, USA), and quantified by QPCR (1.5 nM). The 5' end of each library is phosphorylated and circularized. Then, cyclic amplification is performed to generate DNA nanoballs. Finally, these DNA nanoballs are loaded onto the flow cell for DNBSEQ-T7 gene sequencing at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China). The entire library preparation process is completed to obtain the final DNA library.

In addition, Bray-Curtis distance was calculated using the vegan package for Principal Coordinate Analysis (PCoA). Linear Discriminant Analysis Effect Size (LEfSe) analysis was used for Kruskal-Wallis test and Wilcoxon rank-sum test, with $p < 0.05$ considered statistically

significant. The LDA score was set to 2.0 to identify features with significant differences between groups. Taxa with an average relative abundance greater than 0.0001 (0.01%) were retained in this study. Bar charts were visualized using the "ggplot2" package in R software (version 4.0.5) (https://www.r-project.org/).

All data are expressed as mean ± standard error of the mean (SEM). Differences between groups were analyzed using one-way analysis of variance (One Way ANOVA) with GraphPad Prism 7.0 software, and inter-group difference results were obtained via Tukey's test.

9. References

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