Randomized Controlled Trial

Clinical Trial of Ozonated Water Enema for the Treatment of Fibromyalgia: A Randomized, Double-Blind Trial

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Background: The pathogenesis of fibromyalgia (FM) is currently unknown. Many patients with this condition are not effectively treated, and disorders of the intestinal dysbiosis have been identified in patients with FM. This trial aimed to investigate whether ozonated water enema could alleviate the symptoms of FM by improving intestinal dysbiosis in these patients.

Objective: This trial aims to evaluate the therapeutic advantages of ozonated water enema therapy for patients suffering from FM.

Study Design: A single-center, double-blind, randomized controlled trial.

Setting: Department of Pain Management, Shanghai East Hospital, School of Medicine, Tongji University, Shanghai, China.

Methods: This is a randomized, double-blind trial conducted on FM patients (n = 66). The selected patients were randomly categorized into the O_3 and control groups. The patients in the O_3 and control groups received an ozonated and deionized water enema, respectively, at the same dose and frequency. After the treatment, the scores on the numerical rating scale (NRS), widespread pain index (WPI), Hamilton anxiety scale (HAMA), and the Pittsburgh sleep quality index (PSQI) were compared between the 2 groups, as were the doses of duloxetine, to evaluate the treatment effect. Furthermore, the effectiveness of the treatment was assessed by comparing fecal samples from the O_3 group collected before and after treatment with 25 healthy individuals from the physical examination department of Shanghai East Hospital.

Results: The patients in the O_3 group indicated significant relief in pain and reduced NRS, HAMA, PSQI, and WPI scores at each follow-up time point ($P < 0.001$) when compared to the control group. In addition, the patients in the O_3 group used lower doses of duloxetine than did those the control group ($P < 0.001$). Moreover, FM patients treated with ozonated water indicated improvements to their gut microbiome.

Limitations: The trial's findings might be affected by confounding factors, including medicines, diet, and environmental circumstances. Also, this trial was limited by its sample size, and the symptom severity scores (SSS) of the patients at 3 months after treatment at the given follow-up period were not assessed.

Conclusion: This trial confirmed that the symptoms of pain, anxiety, and sleep disorders in FM patients were effectively relieved after treatment with an ozonated water enema. Furthermore, the ozonated water enema was associated with a significant reduction in duloxetine dosage and improved gut microbiome disorder, suggesting that the enema could target disorders related to the gut microbiome and therefore serve as a therapeutic intervention for FM.

Key words: Fibromyalgia, ozone, ozonated water enema, gut microbiome, chronic pain, dysbiosis

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Fibromyalgia (FM) is among the most prevalent types of persistent widespread pain, affecting approximately 2-4% of adults worldwide, and is more frequent in women than men. This condition is characterized by pain, intestinal dysfunction, irritable bowel syndrome, anxiety, depression, physical exhaustion, physical fatigue, sleep disturbance, and cognitive symptoms, thereby causing a significant reduction in the patient's quality of life (1-3). Currently, although FM is treated primarily with medications, such as duloxetine and amitriptyline, many FM patients do not achieve complete pain relief because of adverse reactions to and poor results from these substances, among other reasons (4). The pathogenesis of FM remains unclear, and several hypotheses for it have been proposed, including impaired nociceptive processing in the central nervous system, altered peripheral nociception, and systemic inflammation (5). However, FM development has been associated with central sensitization, weakening of the downlink inhibition system, and abnormal release of neurotransmitters (3). The central sensitization is involved in the complex connection between the gut and the brain; the gut-brain axis, therefore, has become a focus of recent research (6).

Ozone possesses potent oxidation and sterilizing properties because of its excellent sterilization and oxidation and is widely used to treat various diseases. Furthermore, the use of ozone as an adjuvant therapy has been proposed as a novel approach to treat periodontitis (7). Ozone rectal insufflation has been clinically applied to ulcerative colitis, infectious colitis, and other ailments (8-11). As a kind of ozone therapy, the ozonated water enema has the advantages of low cost, convenience, and safety. Many studies have shown that ozone is very safe for the treatment of pain disorders. A German study from 1982 suggests that only 0.7 problems arise for every 100,000 such treatments, and almost all of them are caused by inappropriate administration (12). Therefore, this trial administered ozonated water enemas to FM patients to elucidate the curative effects of O_3 on these patients and the potential therapeutic pathways involved.

Methods

Study Design

Newly diagnosed FM patients who enrolled in the outpatient department of the Shanghai East Hospital between September 2021 and June 2022 were selected for this research. Twenty-five healthy volunteers, matched for age, gender, and body mass index, were included from the health management center of the same hospital. This trial was approved by the ethics committee of East Hospital, Tongji University School of Medicine ([2022] Trial No. 208), and the protocol was registered at the Chinese Clinical Trial Registry (ChiCTR2100052744). All patients and healthy controls (HC) provided signed informed consent before sample collection.

Inclusion Criteria

(1) Being 18-60 years of age, men and women not restricted;
(2) Having FMS and meeting the American College of Rheumatology's 2016 diagnostic criteria while having been diagnosed for > 12 months;
(3) Having a pre-treatment Numeric Rating Scale (NRS) score of ≥ 4;
(4) Voluntarily signing the informed consent form and possessing the ability to cooperate to complete the questionnaire.

Exclusion Criteria

(1) Being unable to complete the scale assessment due to communication barriers;
(2) Having been diagnosed with neuropathic pain or secondary to osteoarthritis, rheumatoid arthritis, trauma, hypothyroidism, malignant tumor, or other diseases;
(3) Suffering from other diffuse soft tissue pain diseases or other types of rheumatism;
(4) Having thalassemia, sickle cell anemia, glucose-6-phosphate dehydrogenase deficiency (fava disease) or toxic diffuse goiter (Graves disease);
(5) Having severe heart, liver, kidney, or metabolic diseases, mental illness, a history of alcohol abuse or drug use, or pregnancy;
(6) Having taken analgesic, sedative, anti-inflammatory, antidepressant, or other drugs within 4 weeks;
(7) Being diagnosed by colonoscopy with inflammatory colopathy, Crohn's disease, or malignant tumor;
(8) Having various reasons not to be treated with colonoscopy and ozonated water enemas.

Sample Size Calculation and Random Grouping

We began by carrying out the pre-experiment. During this stage, 10 patients were included in each group, one given a treatment consisting of an ozonated water

enema and drugs and the other a treatment consisting of deionized water and drugs, with a follow-up of 3 months. According to the comprehensive evaluation of NRS scores, symptoms, and other clinical features, 8 patients in the ozonated water enema + drug treatment group were responsive, compared to 4 responsive patients in the deionized water + drug treatment group. Upon seeing the results, we assumed that the effective rate of the treatment group was 80%, the effective rate of the control group was 40%, and the value of $\alpha = 0.025$ and $\beta = 0.1$, assuming that the rate of loss to follow-up in each group was 20%. The minimum sample size of each group was calculated to be 25, and we planned to collect 33 cases in each group.

Therapeutic Method and Sample Collection

The recruited patients were randomly divided into the O_3 and the control groups. All patients underwent colonoscopies to rule out inflammatory colon disease and other intestinal diseases after enrollment. The O_3 group received the first treatment under colonoscopy. Briefly, 750 mL of ozonated water was infused into the ileocecal region; from the second day onward, the patients were given rectal perfusion of 500 mL of ozonated water once every other day for a total of 7 times. The control group received deionized water enemas with the same dose and frequency. After the treatment, the NRS, widespread pain index (WPI), Hamilton anxiety scale (HAMA), and Pittsburgh Sleep Quality Index (PSQI) scores of the O_3 group were compared with those of the control group to evaluate the treatment effect. The O_3 group's gut microbiomes were analyzed, given treatment with 16S rRNA, and compared with 25 healthy volunteers (Fig. 1).

The fecal samples from the patients and the HC group were all collected fresh at the hospital and were stored at -80°C within 20 minutes of collection.

Ozonated Water Preparation

In accordance with the protocol, the patients in the ozone group underwent a total of 7 medical interventions. The control group received the same volume of deionized water enemas. Germany Carter's original medical ozone instrument was used to prepare ozonated water. Briefly, deionized water was poured into the therapeutic instrument; the parameter was set at 80 μg/L for 10 minutess. Then, the ozonated water was poured into a sterile container for enema treatment. The ozonated or deionized water was prepared by a specialist, sent to the treating physician, and used immediately after preparation.

16S rDNA Amplicon Pyrosequencing

The bacterial V416S rRNA region was amplified using the forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and the reverse primer 806R (5′- GGACTACHVGGGTWTCTAAT-3′). For multiplex sequencing,

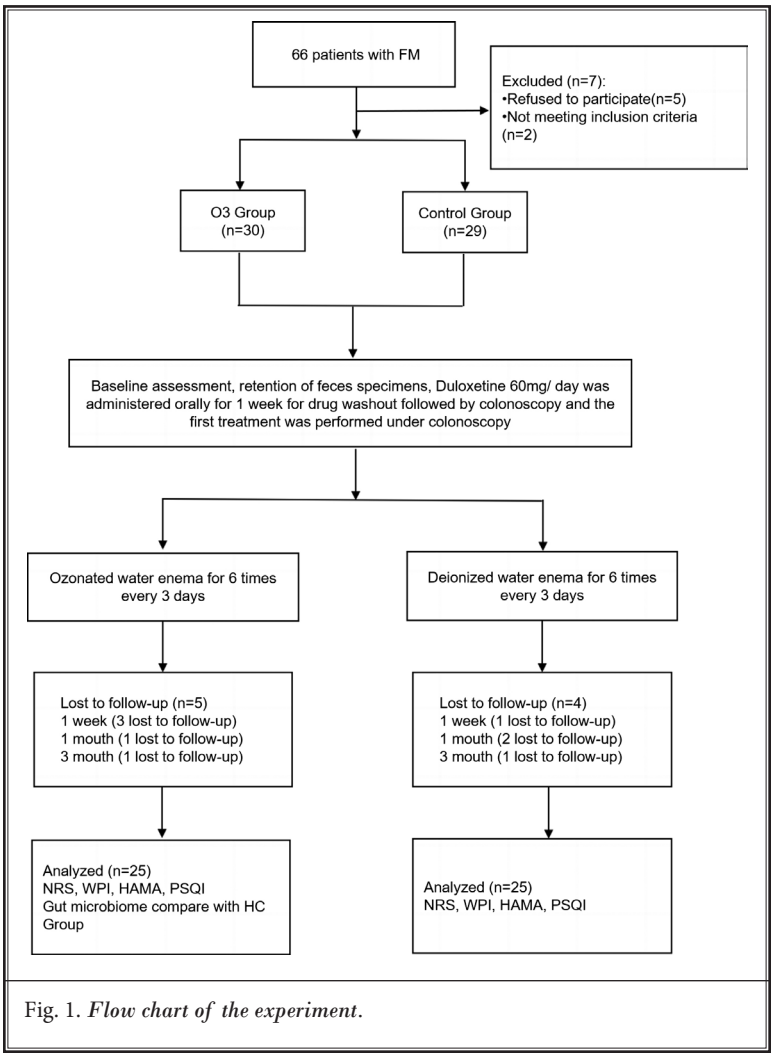

Fig. 1. *Flow chart of the experiment.*

sample-specific 6 bp barcodes were incorporated into the TrueSeq adapters. The polymerase chain reaction (PCR) reaction comprised 25 μL of Phusion® High-Fidelity PCR MasterMix (New England Biolabs, Inc.), 3 μL (10 μM) of each forward and reverse primer, 10 μL of a DNA template, 3 μl of dimethyl sulfoxide, and 6 μL of double-distilled water. The thermal cycling protocol was: initial denaturation at 98°C for 30 seconds, followed denaturation by 25 cycles at 98°C for 15 seconds, annealing at 58°C for 15 seconds, extension at 72°C for 15 seconds, and a final extension at 72°C for one minute. For PCR amplicon purification, Agencourt AMPure XP Beads (Beckman Coulter, Inc.) were used, and they were quantified with the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Fisher Scientific Inc.). The amplicons then underwent PCR amplicon pooling in equal volumes and were subjected to paired-end sequencing using the Illumina NovaSeq6000 platform (Hangzhou Guhe Information Technology Co., Ltd).

Bioinformatics Analysis

The sequence data analyses were conducted using QIIME2™ and R packages (v3.2.0). The Shannon diversity index and beta diversity analysis were assessed using the OTU table in QIIME2™ and principal coordinate analysis, respectively. To examine the variation in microbial communities across samples, nonmetric multidimensional scaling with UniFrac distance metrics was employed. Taxa abundances at various levels (phylum, class, order, family, genus, and species) were statistically compared between samples or groups using the Kruskal test function from the R stats package (metagenomeSeq packages). To identify differentially abundant taxa between the groups, LEfSe (linear discriminant analysis effect size) with default parameters was employed. The output file was further analyzed using the STAMP software package v2.1.3.

Primary Indicators

The NRS pain score is used to evaluate the patients' degrees of pain on the Digital Pain Assessment Scale. Patients rate the degree of pain they feel on a 11-point scale. Zero is no pain, the one-3 level is mild pain, the 4-6 level is moderate pain, and the 7-10 level is severe pain. The patients were required to indicate the level of pain immediately before the treatment and at one week, one month, and 3 months after the treatment. The NRS score is the average pain score over the past 24 hours or week.

Secondary Indicators

1. The widespread pain index (WPI) refers to the number of sites in which the patients experienced pain during the past week. The score is between 0 and 19. The evaluation sites include the left shoulder girdle, left hip, left jaw, upper back, right shoulder girdle, right hip, right jaw, lower back, left upper arm, left thigh, chest, neck, right upper arm, right thigh, abdomen, left forearm, left calf, right forearm, and right calf. The score of pain for each location is one point. Each patient's WPI score was evaluated before and after treatment for one week, one month, and 3 months.

2. The HAMA is used to assess patients' anxiety levels. Employing this scale is a reliable and effective method for measuring the severity of anxiety. The total score is ≥ 29, which may be severe anxiety. At ≥ 21 points, there must be obvious anxiety; at ≥ 14 points, the patient definitely has anxiety; at over 7 points, the patient may have anxiety; if the score is less than 7, there are no anxiety symptoms. The number of pain sites was assessed before treatment began and at one month and 3 months after treatment.

3. The PSQI is used to evaluate the sleep quality of the patients in the past month. This index consists of 19 self-evaluation items and 5 examiner-evaluation items. The nineteenth self-evaluation item and 5 examiner-evaluation items do not contribute to the scoring. A higher score means worse sleep quality. The sleep quality experienced by each patient was evaluated before treatment and at one month and 3 months thereafter.

4. The dose of duloxetine is used to assess the severity of the patient's symptoms. The higher the dose, the more severe the symptoms. The dosage was assessed before the start of treatment and at one week, one month, and 3 months afterward.

Statistical Analysis

IBM SPSS 26.0 (IBM Corporation) was utilized for statistical analysis, and the data were presented as either mean ± SD or median (interquartile range). The Fisher exact or chi-squared tests were employed for analyzing dichotomous data. For normally distributed continuous data, the Student t-test was performed, and for nonparametric ordinal data, the Mann-Whitney U test was conducted. Analysis of variance for repeated measures was used to assess differences in means between and within groups. A statistically significant difference was defined as $P < 0.05$.

Results

Demographic Characteristics of the Cohorts

In this trial, a total of 66 patients were initially recruited. However, 5 patients declined participation, 2 did not meet the inclusion criteria, and 9 were lost to follow-up (5 patients from the O_3 group and 4 patients from the control group). Ultimately, 50 FM patients were enrolled in the trial; of these, 25 received the O_3 treatment, and 25 received the control treatment. Fecal samples from 25 FM patients in the O_3 group were compared with samples from 25 healthy volunteers. Baseline characteristics showed no significant differences between the O_3 and control groups ($P > 0.05$, independent samples t-test, Table 1).

The Patients' Clinical Symptoms Were Improved Significantly by Ozonated Water Enemas

For the purpose of evaluating the therapeutic effect of ozonated water enemas on patients, patients were followed for 3 months so their their pain, mood, and sleep quality could be recorded. The follow-up indicated significantly lower NRS scores for the patients of the O_3 group than for those within the control group ($P < 0.001$, Fig. 2A). Furthermore, decreased WPI scores were associated with the O_3 treatment at all follow-up time points ($P < 0.001$, Fig. 2B). Moreover, the anxiety of the FM patients was relieved to a great extent after the O_3 treatment ($P < 0.001$; $P < 0.001$; $P < 0.001$, Fig. 2C), and the patients' sleep quality improved significantly ($P < 0.001$, Fig. 2D).

Ozonated Water Enemas Significantly Reduced the Dose of Duloxetine in FM Patients

The effects of duloxetine treatment were compared between the 2 groups. After enrollment, both groups received 60 mg of duloxetine per day for a week of drug washout, so there were no differences between the 2 groups' doses of duloxetine before the patients started treatment. During the first week after treatment, the dose of duloxetine used by patients in the O_3 group was significantly lower than that used by the control group ($P < 0.001$, Fig. 3). At the subsequent follow-up time points, the dose of duloxetine used by the O_3 group was increased; however, the overall dose showed a downward trend and was statistically different from that of the control group ($P < 0.001$, Fig. 3).

Ozonated Water Enemas Improved Gut Microbiomes in FM Patients Significantly

To further investigate the mechanism by which trioxane improved FM's clinical symptoms, we analyzed gut flora. First, the alpha diversity among the pre-O_3, post-O_3, and HC groups were compared. Alpha diversity was significantly better in the HC group than in other groups (Fig. 4A). The 3 groups demonstrated significant differences in the composition of their gut microbiomes (Fig. 4B). The pre-O_3 group showed a greater abundance of phyla Actinobacteria, Firmicutes, and Proteobacteria than did the HC group. Conversely, the presence of Bacteroidota was barely detected in the pre-O_3 group (Fig. 4C). Additionally, the pre-O_3 group showed an increased relative abundance of Bifidobacterium, Escherichia-Shigella, Subdoligranulum, Streptococcus, and Ruminococcus gnavus group at the genus level. Conversely, Bacteroides and Prevotella had less relative abundance (Fig. 4D). In addition, the relative abundance of these gut microbiomes in the post-O_3 group was intermediate between the HC and

Table 1. *Demographic characteristics of the cohorts.*

Characteristics	Control Group (n = 25)	O_3 Group (n = 25)	*P*
Age (yr)	34.32 ± 8.62	36.84 ± 10.99	0.371
Gender			
Male	8 (32)	7 (28)	0.758
Female	17 (68)	18 (72)	
BMI, kg/m^2	23.71 ± 1.94	23.79 ± 1.84	0.989
numerical rating scale (NRS)	6.08 ± 1.03	5.8 ± 1.22	0.399
widespread pain index (WPI)	9.04 ± 2.09	8.84 ± 2.11	0.776
Hamilton anxiety scale (HAMA)	21.36 ± 5.56	21 ± 5.18	0.829
Pittsburgh sleep quality (PSQI)	12.92 ± 3.46	12.44 ± 4.85	0.642

Comparisons between the O_3 and control groups.
BMI: body mass index, NRS: numeric rating scale, WPI: widespread pain index, HAMA: Hamilton anxiety scale, PSQI: Pittsburgh sleep quality index.

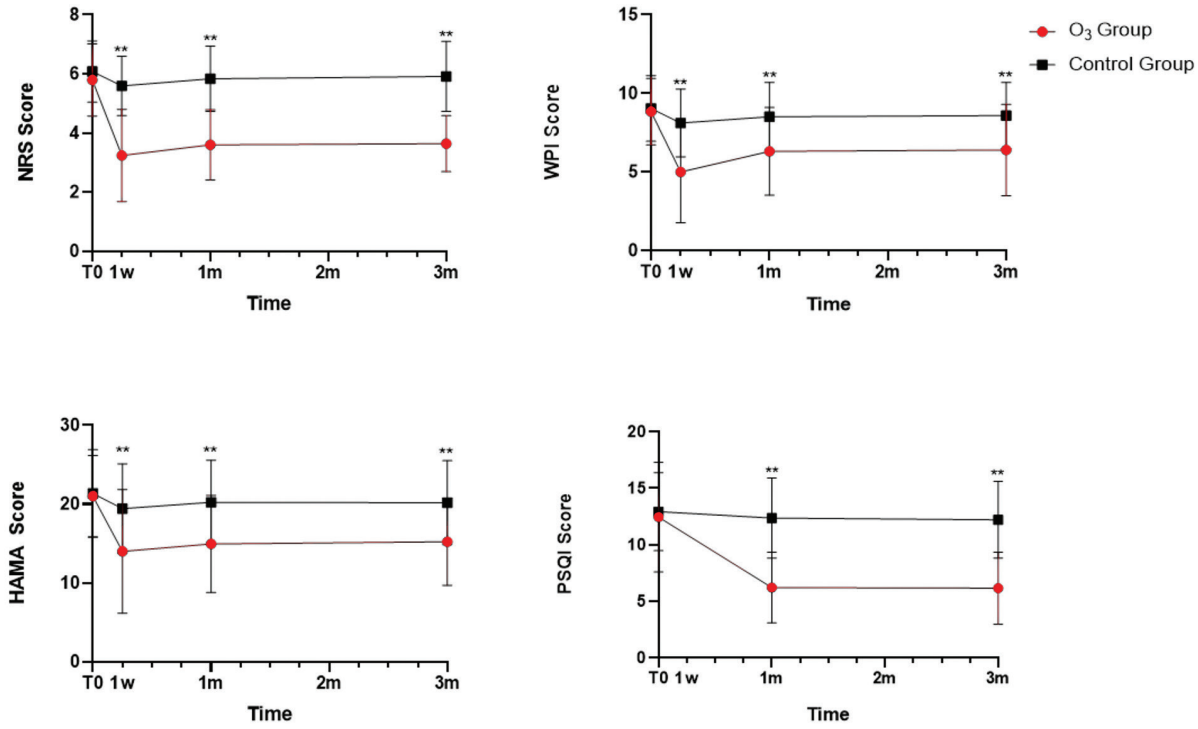

Fig. 2. *The patients' clinical symptoms were significantly improved by ozonated water enemas.*
(A) The NRS score was significantly lower in the O^3 group than in the control group at every follow-up after treatment, according to the independent samples t-test. (B) The WPI score was significantly lower in the O^3 group than in the control group at every follow-up after treatment, according to the independent samples t-test. (C) The HAMA score was significantly lower in the O^3 group than in the control group at every follow-up after treatment, according to the independent samples t-test. (D) The PSQI score was significantly lower in the O^3 group than in the control group at every follow-up after treatment, according to the independent samples t-test.
$^*P < 0.05$, $^{**}P < 0.01$

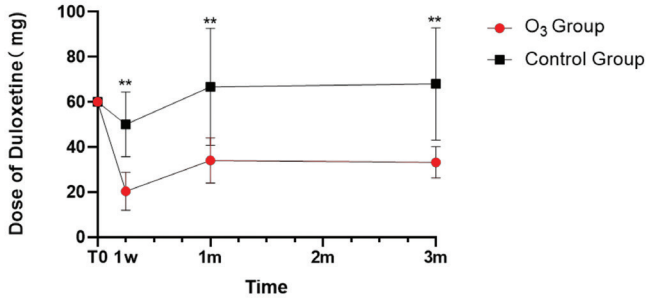

Fig. 3. *Difference in the doses of duloxetine between the O_3 and control groups.*
$^*P < 0.05$, $^{**}P < 0.01$, O_3 group than in the control group.

pre-O_3 groups (Figs. 4C, 4D). The primary taxa that differed among the groups were also identified. LEfSe analysis explored taxonomic abundance and showed significant differences from each group to the others. This analysis revealed that Bacteroidota and Bacteroidia were the most common taxa in the HC group, Firmicute and Bacilli dominated the pre-O_3 group, and Erysipelotrichales and Agathobacter prevailed in the post-O_3 group (Figs. 4E, 4F).

Signature Based on Gut Microbiome Discriminates Among Disease States

The potential value of using the gut microbiota as biomarkers was assessed. At the phylum level, it was revealed that Desulfobacterota, Patescibacteria, Proteobacteria, Actinobacteriota, Bacteroidota, and Firmicutes could be zoned to separate the FM patients from the HC group with an area under curve (AUC) of 0.75 (Figs. 5A, 5B). At the gene level, Faecalibacterium,

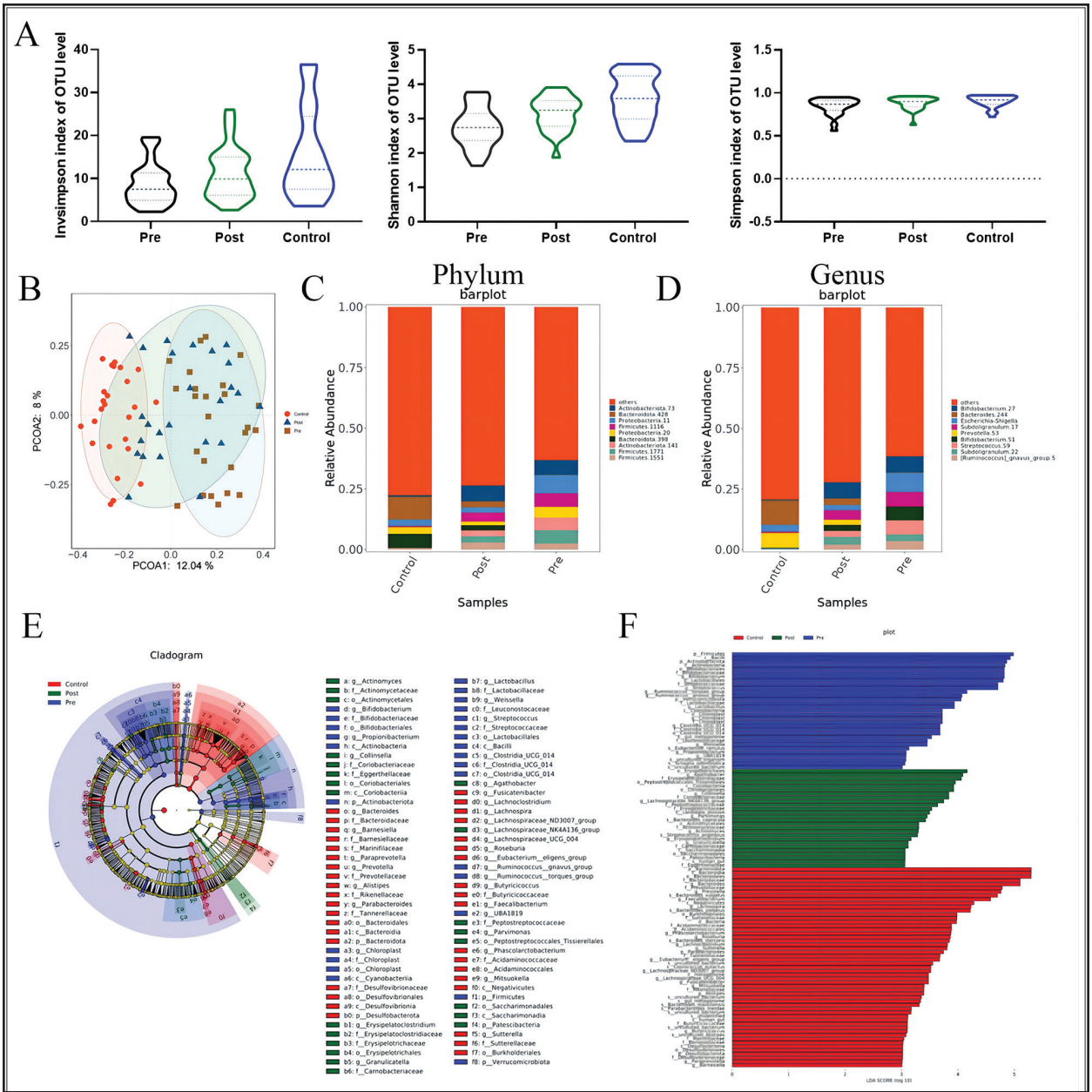

Fig. 4. *Comparison of the composition of the intestinal flora among the post-O^3, pre-O^3, and HC groups.*
(A) Comparison of Invsimpson, Shannon, and Simpson's index among the 3 groups.
(B) PCoA based on an unweighted UniFrac matrix showed that the overall gut microbiome composition differed among the 3 groups.
(C) Analysis of intestinal flora at the phylum level.
(D) Analysis of intestinal flora at the gene level.
(E-F) LDA effect size analysis revealed significant differences in gut flora among the 3 groups.
Post: post-O_3, Pre: pre-O_3, Healthy control: HC

Roseburia, Ruminococcus torques group, etc., have a high predictive value for FM with an AUC of 0.69 (Figs. 5C, 5D). Therefore, the gut microbiome could be used for the diagnosis of FM.

Discussion

This randomized, double-blind trial examined the effect of ozonated water enemas on FM patients.

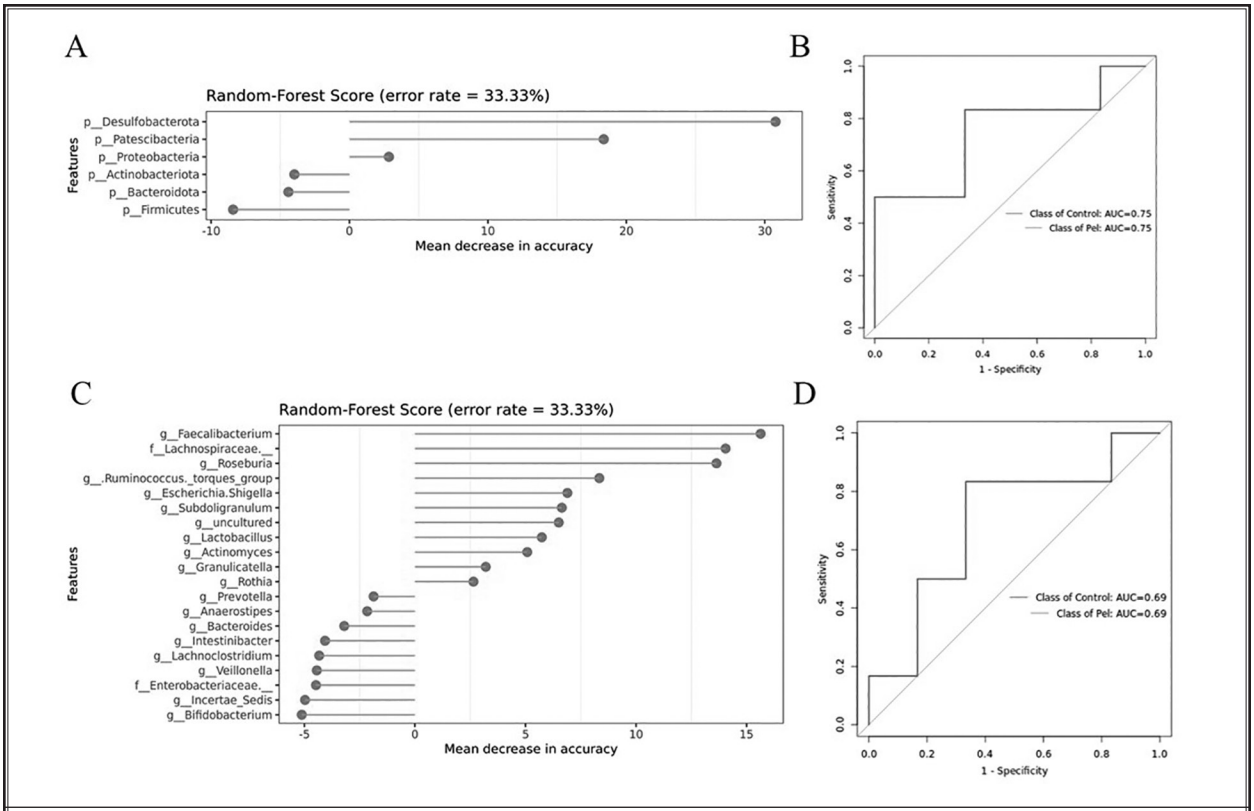

Fig. 5. *Signature based on gut microbiome discriminates among disease states.*
Randomized forest intergroup prediction of intestinal flora at the phylum level (A) and the genus level (C). The classification performance of the multivariable logistic regression model, using the relative abundance of FM-associated genera, was evaluated by calculating the area under the ROC curve at the phylum (B) and genus levels (D), respectively.

The O_3 group indicated significant improvements in gut microbiomes, clinical symptoms, mood, and sleep quality. This result suggests that ozonated water enemas can significantly reduce the clinical symptoms of FM patients. Furthermore, it was revealed that gut microbiome dysbiosis in FM patients was characterized by reduced bacterial diversity and altered relative abundances compared to the HC group. However, after the O_3 treatment, a significant improvement in the diversity of the gut microbiome was also observed in FM patients. Moreover, there was a significant reduction in the difference between the FM patients and the HC group and a marked improvement in symptoms. Thus, the occurrence and development of FM are closely related to the gut microbiome disorder, which could be balanced by the bactericidal effect of O_3.

The pathogenesis of FM is still unclear. Therefore, pharmacological interventions are the mainstay of treatment for the condition. Recent studies, however, have found that duloxetine causes a significantly higher incidence of serious psychiatric adverse events than do placebos in adolescents with FM (13). A new treatment for FM is thus urgently needed. In recent years, central sensitization is considered to be the main pathogenesis of FM. Central sensitization occurs mainly because of abnormal neurotransmitters, and the gut is the main source of many neurotransmitters in the body, so the "gut-brain axis" has gradually entered our field of vision. Minerbi et al compared the microbiomes of 77 women with FM and 79 controls using 16S rRNA gene amplification and whole-genome sequencing. The analysis revealed that 19 kinds of gut microbiomes differed between the FM patients and the control group (2). Marc Clos-Garcia et al (14) found that bacterial diversity was decreased in study patients with FM; the abundance of the genera Bifidobacterium and Eubacterium was significantly reduced in these patients, and serum metabolome analysis showed

changes in glutamate and serine levels, which suggests changes in the metabolism of neurotransmitters. This trial suggests that the onset and development of FM are closely related to the gut microbiome. Our previous study also confirmed that there was a link between FM and intestinal flora and found that fecal bacteria transplantation (FMT) could significantly improve clinical symptoms in patients with FM. Moreover, FMT can increase patients' levels of plasma neurotransmitters, which further provides strong evidence that FM can be treated by improving the condition of the patient's intestinal flora by improving the central sensitization in turn (15).

As the research into the medicinal properties of ozone has increased, ozone is now widely used for treating various pain disorders (12). The local effect of O_3 therapy is reflected in the influence of trioxygen on the patient's intestinal tract. Strong ozone oxidation can directly kill pathogens, equalize the gut microbiome, and improve intestinal infection symptoms such as diarrhea. These effects might be how trioxane enemas improve the clinical symptoms of FM. The systemic effects of O_3 therapy are reflected in the reaction of trioxygen with intestinal mucin and secretions and the generation of reactive oxygen species (ROS) and lipid peroxides (16). ROS are not absorbed in the blood. Meanwhile the lipid peroxides decay gradually in the intestinal tract, enter the circulation, play an antiviral role, improve blood circulation, promote metabolism, regulate immunity, and improve systemic antioxidant capacity (17,18). A study showed that intra-rectal administration of ozone could immediately stimulate the turnover of the epithelial layer in the colon (19). The role of this effect of O_3 on FM remains to be studied.

In addition, another study showed that clinical symptoms of FM were positively correlated with abnormal activation of microglia (20). Microglia can produce and release various pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, etc. (21), which trigger pain through different regulatory mechanisms. The literature suggests that intestinal flora disturbances can induce microglia over-activation (22). It was observed that when mice were treated with IgG from FM patients, their sensitivity to mechanical and cold stimuli increased, as did the reactivity of the injurious fibers in the mice's skin nerves. These results suggest strongly that something is wrong with FM patients' immune systems (23). Therefore, the immunomodulatory effect of ozone may be the one of the main mechanisms for the treatment of FM and balancing the gut microbiome.

Interestingly, in the control group, the clinical symptoms of FM patients were also improved to some extent, although less obviously than in the O_3 group, and the time of symptom improvement was very short. We speculated that the first reason was the therapeutic effect of duloxetine on FM, and the placebo effect could not be ignored in FM patients. The researchers found that placebos also had some effect in improving pain, fatigue, and sleep in patients with FM (24). In another study, placebo and electroacupuncture were associated with nearly the same effect on elderly people with chronic low back pain, and the placebo effect was more significant, further highlighting the role of the placebo effect in chronic pain diseases (25). However, such excellent efficacy was not achieved in our experiment, possibly because FM patients have been ill for a long time and the pathogenesis is unclear. In addition, FM patients have experienced both physical and mental pressure in multiple hospitals over a long period of time, so the expected efficacy of the disease is reduced. This finding is consistent with those of another study: as the duration of FM patients' disease increases, the disease becomes more and more difficult to improve through active treatment, placebo or other external environmental changes, which forces doctors to find new ways to save these patients with long-term disease (24).

Limitations

The limitations of this trial include the following: 1) The findings might be affected by confounding factors, including medicines, diet, and environmental circumstances. 2) The sample size was small, and the follow-up period was short, which might have impacted the results of the experiment. 3) The symptom severity score (SSS) of the patients at 3 months after treatment at the given follow-up period was not assessed.

Conclusion

In summary, this trial demonstrated that FM was closely related to gut microbiome disorders and that ozonated water enemas could effectively improve FM patients' clinical symptoms and gut microbiomes. Additionally, the results suggest that the gut microbiome can be used as a diagnostic marker for FM. Further research on the role of intestinal microbiota in FM development and the specific mechanism by which O_3 therapy works in FM are required.

Data Availability Statement

All data generated in this trial are publicly available. The 16s RNA sequence data can be found in the NCBI database under accession number PRJNA1004835.

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