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The Value of PHQ-9 and GAD-7 for Screening Emotional Disorders in IBS-D and the Specificity of the Gut Flora Associated with Emotional Comorbidity: Preliminary Findings

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Background: To identify irritable bowel syndrome with diarrhea (IBS-D) combined with anxiety and/or depression through a psychological screening tool and to further explore the relationships between patients with comorbidities and gut microbiota.

Methods: The GAD-7, SAS, PHQ-9 and SDS were administered to evaluate anxiety and depression. Faeces were subsequently collected from 44 patients with emotional disorders (IBS-EDs), 22 patients without emotional disorders (IBS-nEDs) and 18 healthy controls (HCs) via 16S rRNA sequencing, depending on the participants' wishes. The differences in gut microbiota among different groups were analysed. Spearman analysis was conducted at the genus level and was based on psychological assessment scores. Patients with IBS-D were recruited from December 2020 to November 2022.

Results: This study included 124 outpatients with IBS-D. According to the GAD-7 and SAS scores, 40.3% and 19.3% of the participants, respectively, had anxiety ($P < 0.05$). Similarly, a significantly greater percentage of participants had depression according to the PHQ-9 than according to the SDS (61.3% vs 33.1%) ($P < 0.05$). Overall, approximately 66.1% of the participants had emotional disorders (anxiety and/or depression) according to the GAD-7 and PHQ-9. Correlation analysis revealed that the abundances of *Eubacterium_hallii_group, Monoglobus* and *Lachnoclostridium* were closely related to the PHQ-9 scores and that the abundances of *Subdoligranulum* and *Holdemanella* were closely related to the GAD-7 scores.

Conclusion: In comparison to the SAS and SDS, both the GAD-7 and PHQ-9 identified a greater number of individuals with emotional disorders within the IBS-D population. Furthermore, our findings demonstrated that *Lachnoclostridium* is not only a biomarker for IBS-ED patients but also that its abundance changes are related to PHQ-9 scores, which may provide insights for further brain gut investigations.

Keywords: depression, anxiety, IBS-D, 16S rRNA

Introduction

Irritable bowel syndrome (IBS) is the most prevalent functional bowel disorder. It is characterized by abdominal pain, abdominal distention, and bowel habit abnormalities.[1,2] The prevalence of IBS is reported to be 9.6% (9.5–9.8%) in Asia, and 5.8% (5.6–6.0%) in the Africa and Middle East.[2] Although the country-specific, region-specific, and global prevalence rates of IBS remain unknown, a recent study to determine the prevalence rate of IBS in Chinese university students (8.6%) reported a rate similar to that in a previous study.[3] IBS is classified into four distinct types based on the Rome IV diagnostic criteria: IBS with diarrhea (IBS-D), IBS with constipation, IBS characterized by alternating constipation and diarrhea, and undefined IBS.[4] Among these subtypes, IBS-D is the most common, approximately

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40%-60% of the IBS population in China.[5] However, because IBS-D is essentially a diagnosis of exclusion, its exact pathophysiological mechanism remains unknown. Therefore, multiple laboratory tests, investigations, and prescription drug treatments that only target symptoms could cause a significant economic burden and psychological pressure for patients.

In clinical practice, psychological factors are important because they cause patients with moderate to severe IBS to seek medical treatment. Regrettably, the Rome criteria used for diagnosing IBS are mostly gut-restrictive, ignoring the fact that most patients with IBS-D meet the diagnostic criteria for anxiety or depressive disorders. And most patients with anxiety and depression disorders have IBS-D symptoms.[6] On the one hand, symptoms of IBS-D, such as diarrhea, abdominal pain and discomfort, and distress with disease control, may contribute to the exacerbation of emotional disorders. On the other hand, long-term anxiety and depression may affect the operation and sensitivity of the intestine, as well as the quality of life.[7,8] In other words, the quality of life of patients with IBS-D was significantly affected by psychiatric symptoms, and the more significant the emotional disorders, the wider the dimensions of quality of life were affected.[9] Hence, diagnosing the condition from the patient's perspective in daily clinical practice as soon as possible is important. The Generalized Anxiety Scale 7 (GAD-7),[10] 9-Item Patient Health Questionnaire (PHQ-9),[11] Self-rating Anxiety Scale (SAS),[12] and Self-rating Depression Scale (SDS)[13] are the scales most commonly used by nonpsychiatrists to assess anxiety and/or depression in outpatient settings. We reviewed the relevant literature and found that previous studies have used SAS/SDS to assess anxiety/depression levels in IBS patients.[14,15] However, recent guidelines have advocated for the implementation of the PHQ-9 for assessing depression and the GAD-7 for evaluating anxiety.[16] Compared with the GAD-7 and PHQ-9, the SAS and SDS had lower sensitivity and more missed diagnoses in nonpsychiatric patients.[17] The PHQ-9 and GAD-7 are more practical for use in clinical settings and can be completed within a few minutes. Therefore, the use of these scales has less influence on the total visit time and allows patients to cooperate more easily.[18] However, there has been a notable lack of studies comparing the effectiveness of the GAD-7/PHQ-9 and SAS/SDS in screening patients with IBS-D who also suffer from comorbid depression and/or anxiety.

Regarding the potential pathogenesis of IBS-D, on the one hand, strong evidence supports that psychological factors have the potential to disrupt intestinal mucosal integrity, modify gut microbiota composition, impair mucosal barrier function, and modulate immune responses.[19,20] Moreover, anxiety and depressive symptoms may exacerbate gastrointestinal and extra-gastrointestinal symptoms by altering visceral hypersensitivity and the intestinal microenvironment, influencing the microbiota-intestinal-brain axis.[21,22] On the other hand, in several studies exploring the effects of fecal microbiota transplantation on IBS-D patients with anxiety and depression behaviors have found that the patient's anxiety and/or depression behavior gradually improved with fecal microbiota transplantation treatment.[8,23] Moreover, the clinical symptoms quality of life had also been relieved significantly.[23] Above all, the microbiome has commonly been observed as a possible link between IBS-D and psychological disorders, and this relationship is gaining ground. However, few studies in China have classified the IBS-D population into anxiety–depression (emotional disorders) and nonanxiety–depression (nonemotional disorder controls), and correlations between psychological alterations and microbial changes in IBS-D patients are less discussed.

Regarding the potential pathogenesis of IBS-D, a brain–gut interaction was identified in a prospective study of IBS with anxiety and/or depression and control individuals without both conditions. At the end of the study, those with IBS at baseline reported significantly higher levels of anxiety and/or depression. Similarly, people with higher baseline levels of anxiety and depression were significantly more likely to develop IBS.[24] Furthermore, strong evidence supports that the microbiome is an indispensable participant in gut–brain communication, and the microbiome–gut–brain axis has been proposed.[25] With the rapid advancement of 16S rRNA amplicon sequencing technologies, numerous studies have uncovered notable disparities in the abundance, composition, and functional characteristics of the gut microbiome between HCs and individuals suffering from IBS-D.[26,27] The microbiome has commonly been observed as a possible link between IBS and psychological disorders, and this relationship is gaining ground.[28] However, few studies in China have classified the IBS-D population into anxiety–depression (emotional disorders) and nonanxiety–depression (nonemotional disorder controls), and correlations between psychological alterations and microbial changes in IBS-D patients are less discussed.

Hence, we aimed to 1) identify patients with IBS-D with emotional disorders through a psychological screening tool and 2) further explore the associations between different gut microbiota and of the presence of emotional disorders.

Methods

Participants

We recruited outpatients by posting on hospital social media sites and fliers from December 2020 to November 2022. The enrolled subjects were aged 18–70 years, and patients had to meet the Rome IV criteria, and had a history of the following stool traits: a Bristol scores of 6–7 at least 25% of the time; and a Bristol score of 1–2 for less than 25% of the time. Participants were excluded if they 1) had a history of severe intestinal organic lesions; 2) had one or more of the warning symptoms; 3) had other severe medical conditions, such as cardiovascular disease, hepatic and renal function disorder; or 4) cognitive disorders or had clearly diagnosed psychosis.

This study adheres to the pertinent guidelines outlined in the Declaration of Helsinki. Prior to their participation, all patients provided written informed consent. The data collection procedure was discussed and approved by the Ethics Committee of Beijing Hospital of Traditional Chinese Medicine, Capital Medical University (Approval No. 2020BL02-050-01).

Sample Size

The cross-sectional study is to investigate the incidence of emotional disorders (comorbidities) in IBS-D patients in the Beijing area (infinite populations). Based on previous experience and a literature review,[29] the predicted standard deviation was 25%, a two-sided test was needed, α was 0.05, the allowable error was 5%, and the sample size was calculated to be 97. To ensure reliability, this trial was expanded to 124 participants.

Assessment Instruments

GAD-7

The GAD-7 serves as an effective screening instrument for identifying generalized anxiety disorders.[10] For GAD-7, scores of 3, 2, 1, and 0, corresponding to "nearly every day", "more than half the days", "several days", and "not at all", respectively. The scores for the 7 questions are summed to determine the total scores. Scores of 0–4 indicate normal anxiety, scores of 5–9 indicate mild anxiety, scores of 10–14 indicate moderate anxiety, and scores of 15–21 indicate severe anxiety.

PHQ-9

The PHQ-9 is a screening tool used to evaluate depression.[11] For PHQ-9, scores of 3, 2, 1 and 0, corresponding to "nearly every day", "more than half the days", "several days", and "not at all", respectively. Higher scores indicate greater anxiety symptoms. Scores ≥ 15 are considered to indicate severe anxiety, scores ≥ 10 are considered to indicate moderate anxiety, and scores ≥ 5 are considered to indicate mild anxiety.

SAS

The SAS is also a screening tool that reflects anxiety disorder.[12] The aggregate scores of the 20 items is multiplied by 1.25, with a higher score indicating more severe levels of anxiety. Scores of > 72, 63–72, and 53–62 are considered to indicate "severe", "moderate", and "mild" anxiety, respectively.

SDS

The SDS serves as a valuable instrument for the assessment of depressive disorders.[13] Patients perform a self-assessment, scoring each item on a 4-point scale. For the SDS, higher scores indicate more severe depression. Grades 3, 2 and 1 correspond to scores of >70, 61–70, and 50–60, respectively.

16S rRNA Sequencing

Stool samples were collected randomly from individuals with IBS-D and HCs. The minimum mass required was 1.0 g per tube. All stool samples were stored at low temperatures and subsequently transported to Majorbio in Shanghai, China, for

further analysis. Following the manufacturer's guidelines, microbial DNA was extracted from the fecal samples. The concentration and purity of the DNA were assessed using a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The hypervariable regions V3-V4 of the bacterial 16S rRNA gene were amplified using the primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplification was conducted with an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). Following purification and quantification, the samples were sequenced on the Illumina MiSeq platform in accordance with established protocols.

The harvested reads were subsequently stored and analyzed using the platform www.majorbio.com. To assess microbial richness in each sample, the Ace, Chao, and Sobs indices were calculated. In addition, the Shannon and Simpson indices were employed to evaluate microbial diversity. Hierarchical clustering and principal coordinate analysis (PCoA) were conducted at the level of all OTUs, utilizing Bray-Curtis distances as the basis for the analysis. Furthermore, species difference analysis was performed to discern the variations in microbial composition between groups and to identify microorganisms that exhibited significant differences.

Statistical Analysis

Statistical analysis was conducted utilizing SPSS version 22.0. To assess consistency among estimators, reliability analysis was carried out employing the kappa statistic. In the context of microbiota analysis, Welch's *t*-test was applied for the calculation of diversity indices. Additionally, to ascertain differences in taxa between the groups, the Mann–Whitney *U*-test was utilized. A *p*-value of less than 0.05 was deemed indicative of statistical significance.

Results

Section One

Emotional Disorders Evaluated by Four Self-Rating Scales

After questionnaires with invalid or incomplete answers and those from nonqualified respondents were excluded from the 210 IBS patients screened, data from 124 IBS-D participants were analysed. The percentages of participants diagnosed with anxiety according to the SAS and GAD-7 were 19.4% and 40.3%, respectively. The number of patients diagnosed with anxiety based on the GAD-7 assessment was notably greater than the number diagnosed using the SAS ($P < 0.05$). Similarly, the PHQ-9 revealed a significantly higher percentage of participants with depression than did the SDS (61.3% vs 33.1%) ($P < 0.05$) (Table 1).

A fair level of agreement was noted between the SAS and GAD-7 for diagnosing anxiety, as evidenced by a kappa coefficient of only 0.31. Furthermore, the SDS and PHQ-9 demonstrated a kappa coefficient of 0.24, which also reflects a similar level of fair agreement between these scales in the assessment of depression.

Table 1 Anxiety and Depression Evaluated by Different Self-Rating Scales

	GAD-7		SAS		PHQ-9		SDS	
	n	%	*n*	%	*n*	%	*n*	%
Without anxiety[a]	74	59.7%	100	80.6%	—	—	—	—
Anxiety total[b]	50	40.3%	24	19.4%	—	—	—	—
Low level	38	30.6%	19	15.3%	—	—	—	—
Moderate level	8	6.5%	5	4.1%	—	—	—	—
High level	4	3.2%	0	0.0%	—	—	—	—
Without depression[c]	—	—	—	—	48	38.7%	83	66.9%
Depression total[d]	—	—	—	—	76	61.3%	41	33.1%
Low level	—	—	—	—	50	40.3%	30	24.2%
Moderate level	—	—	—	—	22	17.8%	11	8.9%
High level	—	—	—	—	4	3.2%	0	0.0%

Notes: [a]$P < 0.05$ patients with anxiety assessed by GAD-7 when compared to those by SAS. [b]$P < 0.05$ different anxiety levels assessed by GAD-7 when compared to those by SAS. [c]$P < 0.05$ patients with depression assessed by PHQ-9 when compared to those by SDS. [d]$P < 0.05$ different depression levels assessed by PHQ-9 when compared to those by SDS.

Participants Characteristics

According to PHQ-9 and GAD-7,[30,31] all the participants were classified as having anxiety and/or depression (emotional disorders group, IBS-ED) (82/124, 66.1%) or not having either disorder (nonemotional disorders control group, IBS-nED) (42/124, 33.9%). Age, sex, marital status, BMI, occupation type, education level, monthly personal income or patient characteristics did not significantly differ between the IBS-ED and IBS-nED groups (Table 2).

Positivity Rate for Each GAD-7 and PHQ-9 Item

First, the positivity rate for each item on the PHQ-9 and GAD-7 was analysed considering all 124 participants as a whole. For GAD-7, the positivity rate for Item 1 (Do you feel nervous, anxious or on edge?), was the highest at nearly 70%. The results for the remaining items are shown in Figure 1A. For PHQ-9, the positivity rate for Item 4 (Do you feel tired or have little energy?) was the highest, at more than 80%. The results for the remaining items are shown in Figure 1B. Second, because Item 1 on the GAD-7 and Item 4 on the PHQ-9 had the highest scores, we further compared the positivity rates and total scores differences between the IBS-ED and IBS-nED groups. The total scores of the PHQ-9 and GAD-7 exhibited significant differences between the two groups for the corresponding categories of 1, 2, and 3, namely "several days", "more than half the days", and "nearly every day", respectively (all $P < 0.05$) (Figure 1C–F).

Clinical Manifestations

To evaluate clinical symptoms, this study also used the professional IBS Symptom Severity Score (IBS-SSS), which was validated by the Rome IV committee and has high reliability, validity, and sensitivity in assessing the severity of symptoms in patients with IBS.[32] The total IBS-SSS scores or subscale scores did not significantly differ between the IBS-ED group and IBS-nED groups (Supplemental Table 1).

Table 2 Sociodemographic Characteristics of Patients with Mental Diseases Assessed by GAD-7 and PHQ-9

	IBS-ED (n=82)	IBS-nED (n=42)	*P*
Age ($\overline{X} \pm s$)	39.4 ± 14.1	36.8 ± 14.0	0.330
Sex (n, %)			
Male	35 (42.7)	17 (40.5)	0.814
Female	47 (57.3)	25 (59.5)	
Marital status (n, %)			
Single	29 (35.4)	20 (47.6)	0.187
Married	53 (64.6)	22 (52.4)	
BMI ($\overline{X} \pm s$)	23.0 ± 4.0	23.3 ± 3.9	0.739
Occupation (n, %)			
Mental work	49 (59.8)	28 (66.7)	0.453
Manual work	33 (40.2)	14 (33.3)	
Education, n (%)			
Graduate	26 (31.7)	7 (16.7)	
Undergraduate	47 (57.3)	31 (73.8)	0.164
High school and below	9 (11.0)	4 (9.5)	
Monthly personal income (n, %)			
Less than 5000 RMB	31 (37.8)	16 (38.1)	
5000–8000 RMB	10 (12.2)	8 (19.0)	0.550
More than 8000 RMB	41 (50.0)	18 (42.9)	
Character			
Introversion	46 (56.1)	20 (47.6)	0.371
Extroversion	36 (43.9)	22 (52.4)	

Abbreviations: BMI, body mass index; IBS-ED, IBS-D with emotional disorders; IBS-nED, IBS-D without emotional disorders; RMB, the renminbi is the currency of the People's Republic of China.

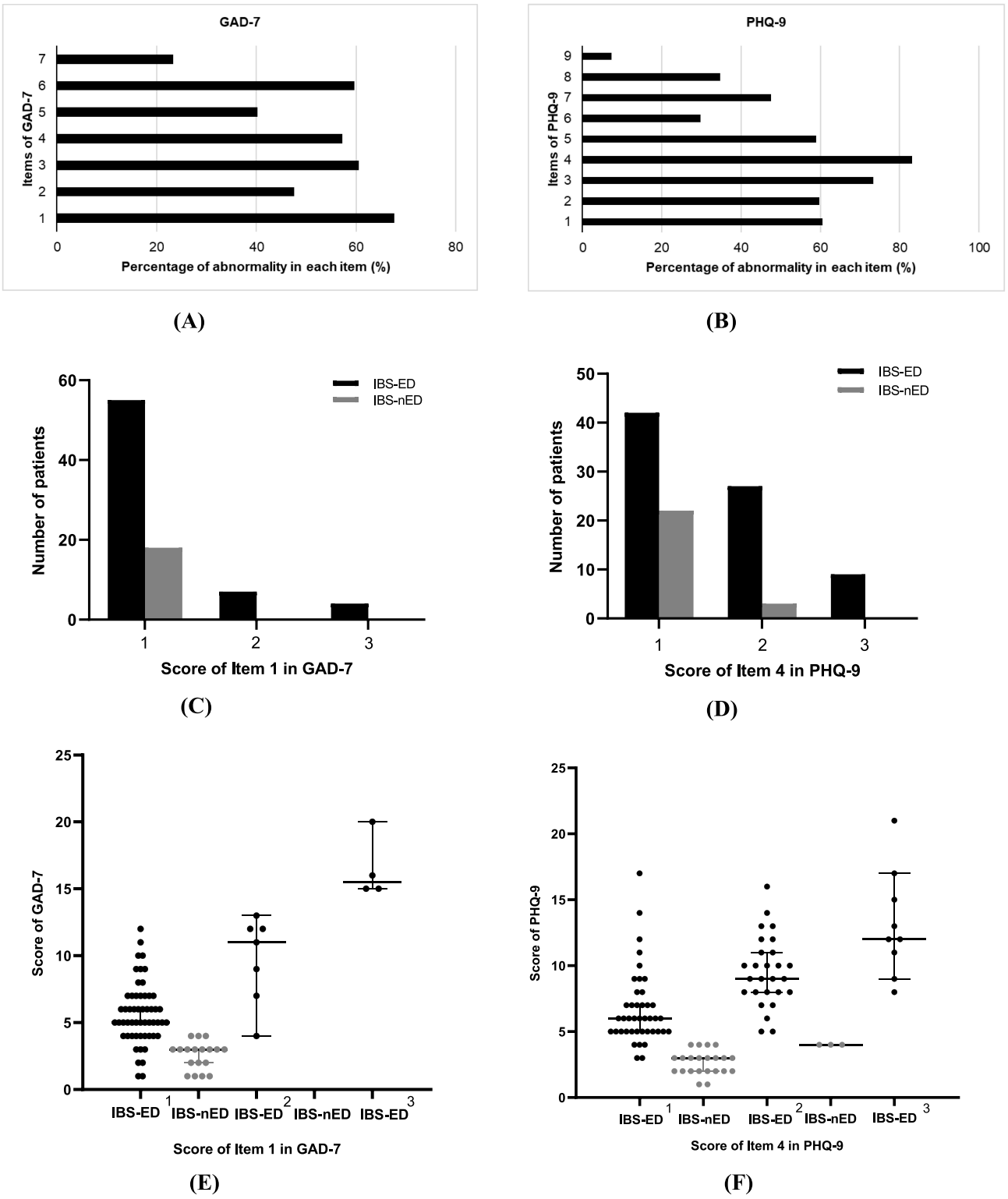

Figure 1 Abnormality in each item of GAD-7 and PHQ-9. **(A)**: The percentage of abnormality of each item in GAD-7. The items were as follows: 1. Feeling nervous, anxious or on edge? 2. Not being able to stop or control worrying? 3. Worrying too much about different things? 4. Trouble relaxing? 5. Being so restless that it is hard to sit still? 6.Becoming easily annoyed or irritable? 7. Feeling afraid as if something awful might happen? **(B)**: The percentage of abnormality in each item of PHQ-9. The items were as follows: 1. Little interest or pleasure in doing things? 2. Feeling down, depressed, or hopeless? 3. Trouble falling or staying asleep, or sleeping too much? 4. Feeling tired or having little energy? 5. Poor appetite or overeating? 6. Feeling bad about yourself or that you are a failure or have let yourself or your family down? 7. Trouble concentrating on things, such as reading the newspaper or watching television? 8. Moving or speaking so slowly that other people could have noticed? Or the opposite being so fidgety or restless that you have been moving around a lot more than usual? 9. Thoughts that you would be better off dead, or of hurting yourself in some way? **(C)**: Number of patients with item 1 of GAD-7 scores which was greater than 0. **(D)**: Number of patients with item 4 of PHQ-9 scores which was greater than 0. **(E)**: Total scores of GAD-7 in different item 1 scores. **(F)**: Total scores of PHQ-9 in different item 4 scores. **Abbreviations**: IBS-ED, IBS-D with emotional disorders; IBS-nED, IBS-D without emotional disorders.

Section Two

Characterization of the Faecal Microbiota in the IBS-ED and HC Groups

For the first part of the study, stool samples were collected from 44 IBS-ED and 22 IBS-nED according to the wishes of the subjects. Eighteen HCs matched for patient sex, age and education level were recruited from the physical examination centre, and stool samples were collected (Supplemental Table 2 for participant characteristics). The Simpson index demonstrates an inverse correlation with the species diversity present in the sample; conversely, the magnitudes of the other indices exhibit a direct proportionality to the levels of diversity or richness. The Shannon index in the IBS-ED group was significantly lower than that in the HC group, as shown in Table 3. However, there was no significant difference in diversity or evenness among the three groups. Following this, we constructed a rarefaction curve and a rank abundance curve based on the clustering results for the OTUs (Supplemental Figure 1A and 1B). Hierarchical clustering and PCoA, utilizing Bray-Curtis distances, were employed at the OTU level to assess the similarities in microbial community structures among fecal samples obtained from the IBS-ED group, IBS-nED group and HC group (Supplemental Figure 2A). The samples from the HC group were unevenly mixed compared with those from the IBS-ED and IBS-nED groups, whereas clear distinctions between the two clusters were not apparent. Similarly, the PCoA plot did not show a clear separation of the fecal samples from these three groups (Supplemental Figure 2B).

At the phylum level (Supplemental Figure 3A), *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria* were the most abundant phyla in the IBS-ED group. *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria* were the most abundant phyla in the IBS-nED group. *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria* were the most abundant phyla in the HC group. The abundance of the most predominant phylum, *Firmicutes*, was significantly lower in the IBS-ED group than in the HC group ($P < 0.05$), whereas the abundance of *Desulfobacterota* was significantly greater in the IBS-ED group than in the HC group ($P < 0.05$). However, there were no significant differences observed in the first ten predominant bacterial genera among the three groups (Supplemental Figure 3B). To facilitate more detailed comparisons between these groups, we employed the Wilcoxon rank sum test. Compared with those of the HC group, the abundances of *Roseburia, Christensenellaceae_R-7_group, norank_f__Coriobacteriales_Incertae_Sedis, Eubacterium_siraeum_group* and *norank_f__norank_o__Clostridia_UCG-014* were significantly lower in the faecal samples of the IBS-nED group, whereas the abundance of *Atopobium* was significantly greater in the faecal samples from the IBS-nED group (Figure 2A). Compared with those from the faecal samples of the HC group, the abundances of *Dorea, Romboutsia, Roseburia*, and *norank_f__Eubacterium_ coprostanoligenes_group* were significantly lower in the faecal samples from the IBS-ED group, whereas the abundances of *Lachnoclostridium* and *Desulfovibrio* were significantly greater in the faecal samples from the IBS-ED group (Figure 2B). Compared with the IBS-nED group, the IBS-ED group presented significantly greater *norank_f__norank_o__Clostridia_UCG-014* content and lower *Streptococcus* and *Atopobium* contents (Figure 2C). To further analyse the statistical significance and biological correlation, we used linear discriminant analysis effect size (LEfSe) to identify statistically significant biomarkers. The results revealed that the biomarkers of IBS-ED patients included *Lachnoclostridium* and *Desulfovibrio* (Supplemental Figure 4A and 4B).

Associations Between Psychological Characteristics and Variations in Microbiota Composition in Patients with IBS-ED

We included two variables related to psychological abnormalities (GAD-7 and PHQ-9) that may have been related to the

Table 3 Comparison of Alpha Diversity Index Between IBS-D and HCs

Group	Sobs	Shannon	Simpson	Ace	Chao
HCs	81.28 ± 18.92	2.82 ± 0.45	0.11 ± 0.06	91.96 ± 21.27	89.35 ± 19.71
IBS-ED	70.84 ± 19.30	2.55 ± 0.49*	0.15 ± 0.09	80.20 ± 21.54	81.44 ± 24.18
IBS-nED	72.77 ± 17.91	2.61 ± 0.36	0.13 ± 0.05	82.04 ± 18.56	81.33 ± 20.31

Note: compared with the HCs group, the difference between groups is statistically significant, *indicate $P < 0.05$.
Abbreviations: HCs, healthy controls; IBS-ED, IBS-D with emotional disorders; IBS-nED, IBS-D without emotional disorders.

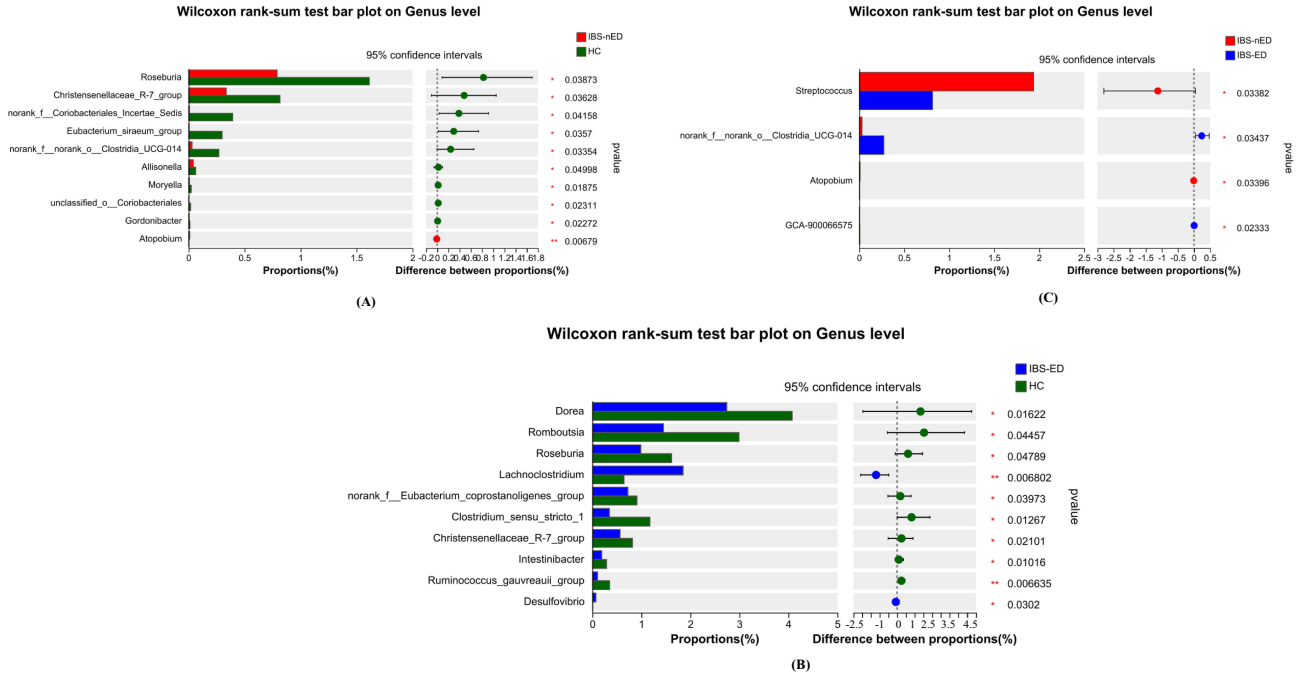

Figure 2 The barplots showed the relative abundance of genera enriched between groups. **(A)**: Taxonomic composition of IBS-nED and HC samples at genus level; **(B)**: Taxonomic composition of IBS-ED patients and HC samples at genus level; **(C)**: Taxonomic composition of IBS-nED and IBS-ED patients at genus level.
Abbreviations: HCs, healthy controls; IBS-ED, IBS-D with emotional disorders; IBS-nED, IBS-D without emotional disorders.

microbial signature in the 44 IBS-ED patients (Supplemental Figure 5). The GAD-7 and PHQ-9 scores were significantly correlated with microbial structure (all $P < 0.05$) (Supplemental Table 3).

Additionally, we investigated the relationships between the composition of the fecal microbiota and clinical metadata using Spearman correlation coefficient (Figure 3). Significant correlations were detected between the relative abundance of *Eubacterium_hallii_group* and the PHQ-9 scores (r= 0.365, P= 0.015), the relative abundance of *Monoglobus* and the PHQ-9 scores (r= 0.309, P= 0.042), the relative abundance of *Lachnoclostridium* and the PHQ-9 scores (r= 0.323, P= 0.032), the relative abundance of *Subdoligranulum* and the GAD-7 scores (r= −0.340, P= 0.024), and the relative abundance of *Holdemanella* and the GAD-7 scores (r= −0.311, P= 0.040).

Discussion

In the first part of this study, two distinct self-assessment scales, namely the SDS and SAS, along with the PHQ-9 and GAD-7, were utilized to evaluate levels of depression and anxiety. Compared with the SDS and SAS, the PHQ-9 and GAD-7 identified significantly greater percentages of patients with depression and anxiety, respectively. This study represents the inaugural effort to evaluate the effectiveness of the PHQ-9/GAD-7 and SDS/SAS as screening instruments for emotional disorders among Chinese patients with IBS-D in an outpatient setting. The corresponding results may guide the clinical screening of emotional disorders in IBS-D patients in nonpsychiatric outpatient clinics in hospitals. The results of the 16S rRNA sequencing conducted in the second part of the study unveiled alterations in gut microbial composition that are linked to psychological and emotional symptoms in the IBS-ED group. These findings might offer valuable insights for future investigations into the mechanisms underlying the interactions between the brain and the gut.

In clinical practice, the available anxiety assessment scales include the GAD-7, General Hospital Anxiety Subscale (HADS-A), SAS, and Hamilton Anxiety Rating Scale (HAMA). The depression assessment scale includes the PHQ (including the PHQ-2 and PHQ-9), the depression subscale of the General Hospital Anxiety and Depression Scale (HADS-D), the SDS, and the Hamilton Rating Scale for Depression (HAMD). To select suitable screening scales, we conducted a literature review and comparison. First, self-rating scales are powerful tools to help gastroenterologists identify emotional disorders in IBS-D. However, non-psychiatrists do not have corresponding qualifications assessed or

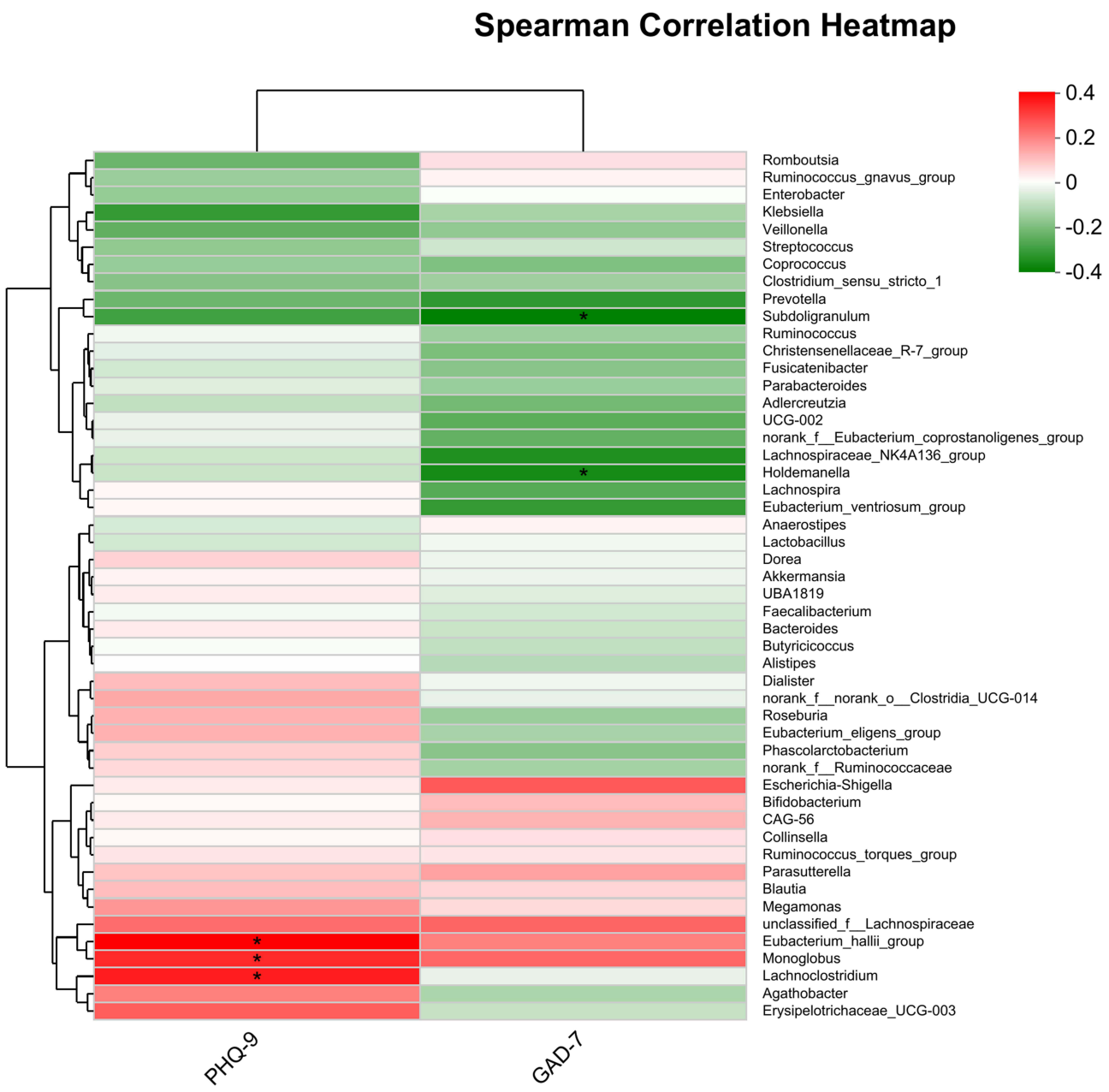

Figure 3 Heatmap of correlations between abundances of genera and clinical scales. Positive and negative correlations are shown in red and green blocks, respectively. *P< 0.05.

used the non-self-rating scales. Therefore, we did not use the HAMA or HAMD. Second, the PHQ-2,[33] also known as the "two-question screening", consists of the first two questions of the PHQ-9 and is simpler but less accurate than the PHQ-9. Third, the GAD-7 and PHQ-9 assessments evaluate the diverse symptom profiles associated with anxiety and depressive disorders, respectively. In contrast, the subscales of HADS (HADS-A and HADS-D), primarily emphasize the emotional dimensions of anxiety and depression, deliberately omitting items that assess somatic symptoms.[34] Compared with the HADS-D, the PHQ-9 has fewer items and is simpler and less time-consuming, and its sensitivity and positive predictive value in IBS patients are greater than those of the HADS-D.[30] Although HADS-A and GAD-7 scores were shown to be highly correlated in a population of IBS, modifying the HADS-A cut-off score substantially affects its sensitivity and specificity. Nevertheless, the difference in cut-off values between the HADS-A and GAD-7 was

not the main purpose of our study. Therefore, we used the PHQ-9 and GAD-7 scales to screen patients for potential emotional disorders.

The proportions of patients with emotional disorders identified using the PHQ-9/GAD-7 scales were significantly different from those identified through the SDS/SAS scales in this study. The SDS or SAS total scores revealed that 33.1% and 19.3% of the patients had depression or anxiety, respectively, whereas the PHQ-9 or GAD-7 revealed that 61.3% and 40.3% of the patients had depression or anxiety, respectively. This discrepancy could potentially be attributed to several factors. First, the detection rates for the SDS or SAS may be comparatively low. Previous studies also revealed a lower percentage of emotional disorders (35% and 28%, respectively) in IBS patients evaluated by the SDS or SAS.[35] Second, because not all 20 items in the SAS and SDS are forward-scoring items, participants are prone to misunderstandings and incorrect choices during self-evaluations, which may lead to excessively high or low comorbidity rates. For example, among the 42 patients, 20 (47.6%) with an SDS scores ≥ 50 were diagnosed with depression, and 17 (40.4%) with an SAS scores ≥ 50 were considered to have anxiety.[36] Conversely, among the 177 patients, 48.6% had anxiety, with a mean SAS scores of 43.54 ± 13.77, whereas only 7.9% had depression, with a mean SDS scores of 37.15 ± 9.43.[15] The latest research findings indicate that major psychosocial problems are present in 23.6%-45.8% of patients with IBS.[29] Anxiety and depression are common psychosocial problems: the pooled prevalence of anxiety symptoms was 39.1% (95% CI: 32.4–45.8), and the pooled prevalence of depressive symptoms was 28.8% (95% CI: 23.6–34.0). In our study, 40.3% of the patients with IBS-D had anxiety, which is consistent with the findings of most previous studies.[37–39] Third, for patients with suspected emotional disorders, the length of the 20-item SDS and SAS might influence the participants' ability to complete these assessments. As the number of items increases, participants find it increasingly challenging to concentrate on the scales, particularly in the bustling environment of the outpatient department. Given the limited number of items in the PHQ-9 and GAD-7 assessments, patients tend to concentrate on all the questions and are able to complete their self-ratings efficiently within a brief timeframe. According to the scores alone, 61.3% of the patients with IBS-D had depression, whereas 66.1% of the patients had emotional disorders (comorbidities). This number was quite similar to the proportion of IBS patients with emotional disorders assessed using the PHQ-9/GAD-7 by other researchers.[31,40] These findings indicate that the PHQ-9 and GAD-7 could serve as optimal screening instruments for identifying potential emotional disorders in Chinese digestive clinics.

As a result of increasing work and life pressures and imbalances in gut microbiota, IBS-D patients with comorbid anxiety and/or depression have attracted increased amounts of attention.[41] Simultaneously, emotional dysregulation is a basic feature found in patients with autism spectrum disorder and attention deficit hyperactivity disorder[42] or bipolar disorder.[43] Therefore, it is important for gastroenterologists, not psychiatrists, to quickly identify and diagnose mood disorders in IBS-D outpatients and refer patients with major depressive disorder or bipolar disorder to psychiatric departments, and to further clarify the relationships between mild to moderate comorbidities and the gut microbiota of patients. The second part of the study also revealed that the diversity of gut microbiota in the IBS-ED group was significantly lower than that in the HC group, but the richness or evenness did not significantly differ between the two groups. Additionally, the beta diversity index did not significantly differ between the groups. At the phylum level, the abundance of *Firmicutes* in the IBS-ED group was significantly lower than that in the HC group, and the abundance of *Desulfobacterota* was significantly greater than that in the HC group. Bacteria did not significantly differ between the IBS-ED group and the IBS-nED group. At the genus level, the abundances of *Dorea, Romboutsia* and *Roseburia* in the IBS-ED group were significantly lower than those in the HC group, whereas the abundance of *Lachnoclostridium* was significantly greater than that in the HC group. Notably, *Streptococcus* and *Atopobium* were significantly less abundant in the IBS-ED group than in the IBS-nED group. Several explanations may exist for these results. First, several studies from Mexico,[44] Japan[45] and China[46] all unanimously suggest that the abundance of *Streptococcus* is significantly increased in IBS-D patients. Kensei Nishida reported that the long-term use of *Lactobacillus gasseri* CP2305-containing tablets may not only improve stress-associated symptoms and clinical symptoms in healthy young adults and in patients with IBS but also decrease the abundance of *Streptococcus* spp.[47] Moreover, our results are similar to those of Yu,[48] who reported that the abundance of *Streptococcus* is decreased in patients with major depressive disorder, and this decrease might play a role in the pathogenic mechanism of depression. Animal studies also confirmed that the abundance of *Streptococcus* was significantly decreased in chronic unpredictable mild stress model mice.[49] While the impact of antidepressant

treatment was correlated with the adjustment of the proportion of probiotics, including *Bifidobacterium* and *Streptococcus*, which mitigate depressive behaviours.[49] Second, *Atopobium* is also a biomarker based on the differences we identified. An analysis of rectal samples revealed a reduction in the concentration of *Atopobium* in tissue samples from IBS-D compared with HCs.[50] However, our results found an elevated *Atopobium* counts in both IBS-ED and IBS-nED groups. It seemed conflictive. Two studies applied the same laboratory setup and observed an increase in relative abundance of *Atopobium* in patients with major depressive disorder.[51,52] Moreover, Lu et al[53] found that among patients with bipolar depression: count of *Atopobium* Cluster was significantly increased relative to HCs, whereas microbial colonization resistance was significantly decreased. Although these parallel findings are intriguing, paradoxically, the abundances of *Atopobium* were lower in the IBS-ED group than in the IBS-nED group, suggesting that the emotional state of patients with IBS-D may not be the direct or sole cause of changes in *Atopobium* abundance. Based on the above findings, we suggest that the changes in *Streptococcus* abundance might partly explain the emotional state of IBS-ED and IBS-nED patients. Simultaneously, the abundances of *Atopobium* in IBS-ED deserve further study in the future.

The results of a subsequent LEfSe analysis suggested that the biomarkers of IBS-ED patients included *Desulfovibrio* and *Lachnoclostridium*. For *Desulfovibrio*, the analysis of the microbiome composition revealed an increase in the abundance of *Desulfovibrio* in IBS patients compared with healthy individuals.[54] *Desulfovibrio spp*. are commensal sulfate-reducing bacteria that are present in small amount of the gastrointestinal tract. Increased concentrations of *Desulfovibrio* spp. *(blooms)* have been reported in IBS.[55] In Japan, the relative abundance of *Desulfovibrio* in the response group decreased after fecal microbiota transplantation.[56] In terms of *Lachnoclostridium*, a recent study that identified microbiome biomarkers of IBS revealed that the abundance of *Lachnoclostridium* is greater in IBS patients than in HCs,[57] which is consistent with our research findings. Animal studies also confirmed that berberine can alleviate visceral hypersensitivity caused by IBS faecal microbiota transplantation, which was related to the enrichment of *Lachnoclostridium*.[58] *Lachnoclostridium* has been shown to be positively correlated not only with abdominal pain and bloating, but also associated with metabolites affiliated to alcohols, bile acids, and derivatives.[59] In this preliminary study, correlation analysis revealed that *Lachnoclostridium* is not only a biomarker for identifying IBS[57] but also correlated with PHQ-9 scores. Overall, although these clinical and animal studies revealed a close association between emotional disorders associated with IBS-D and gut microbiota, further confirmatory studies are essential before these findings can be effectively translated into diagnostic and therapeutic advantages for this patient population.

Some limitations have to be discussed. First, the small sample size of our single-centre study and the insufficient stool samples collected limited the generalizability of the conclusions. Second, we only referred to the standard PHQ-9 and GAD-7 scores grading, without using custom cut-off or higher cut-off values, which might have resulted in the patients' anxiety and depression symptoms being relatively prominent. Third, due to the inability of cross-sectional design to determine causal relationships, more experimental evidence is needed before determining which gut bacteria affect emotions.

Conclusion

This study revealed that PHQ-9 and GAD-7 could be well-validated tools for screening for mood disorders in IBS-D patients in nonpsychiatric clinics for short periods. And there are differences in the abundance of gut flora among IBS-D patients with different levels of anxiety and depression. *Lachnoclostridium* is not only a biomarker for IBS-ED patients, but its abundance changes are related to PHQ-9 scores, which might provide insights for further brain gut investigations.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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