Supplementary material

Materials and Methods

Electroacupuncture (EA)

During the study, only one acupuncturist, who is both the researcher and a medical physician, administered acupuncture to all participants. The acupuncturist completed the acupuncture and moxibustion for doctors' course under The Royal Thai Army Medical Department and Tianjin University of Traditional Chinese Medicine. With over 8 years of clinical practice experience in acupuncture, the acupuncturist is highly qualified. EA was employed using KWD-808I serial impulse electrotherapy with the basic wave adjusted: 1-100 *Hz* with 0.5 *msec*. Six acupoints, Tianshu (ST25), Zusanli (ST36), and Shangjuxu (ST37) on bilateral sides were stimulated. Each participant's skin was cleaned with 75% ethanol before perpendicular insertion of single-use sterile acupuncture needles (EACU; size: 0.25 *mm* in diameter, length: 40 *mm*). Each needle was attached to an electric generator after twisting and obtaining a Qi-sensation of De-qi, which can present as soreness, tingling, fullness, or heaviness. The positive electrode was connected to the negative electrode at ST25 from right to left. For ST36 and ST37, the connection was made from positive to negative in a top-to-bottom direction, following the qi flow along the stomach meridian. An electrical current was input continuously for 30 *min* with stimulation of the ripple wave mode (RIPP) at a frequency of 2/100 *Hz* and the intensity of the current was set at 30% above the patient's electrical sensation. We selected this combination frequency stimulation for maximal analgesic effect[1].

Laxative drug

We will use magnesium hydroxide as a rescue laxative drug for IBS-C patients in phase 2. The usual adult dose of magnesium hydroxide is 800 to 1600 *mg* at bedtime, but this study will use for only 200 *mg*. Magnesium hydroxide is low intestinal absorption and has an osmotic effect which has less effect on abdominal disturbance[2, 3]. The amount of medicine and the frequency of use will be noted. Fewer than five

participants used magnesium hydroxide during the study, but they used it only in the first week of the study protocol. Therefore, for more than two weeks before the secondary collection at post-treatment, they did not use any laxatives because their IBS symptoms did not bother them much. Additionally, during the period after treatment and the one-month follow-up, no participant used the laxative.

IBS-symptoms severity scale scores (IBS-SSS)

This scoring system is a valuable instrument to assess (1) severity, (2) reproducibility and (3) sensitivity to change[4]. The maximum achievable score is 500 which can be interpreted by the level scores (IBS-SSS; less than 75 = no IBS, 75-175 = mild IBS, 175-300 = moderate IBS, and more than 300 = severe IBS). The reduction of more than 50 points in IBS-SSS scores reflects a clinically meaningful improvement, which is considered a significant beneficial therapeutic effect.

IBS-QOL

As described above, beyond the suffering from the symptoms in IBS patients, they always are involved in their quality of life and work productivity. Therefore, when assessing the symptoms or the primary purpose of treatment is to control the symptoms rather than disease cure, it is crucial to measure success in terms of improvement in patient quality of life (QOL).

The IBS-QOL questionnaire is a 34-item instrument developed and validated for measurement health-related quality of life (HRQOL) in non-subtyped IBS patients. The construct validity of the IBS-QOL was assessed and correlated with similar changes from Baseline for IBS-SSS, EQ-5D and average worst abdominal pain. This instrument is used to determine in the part of dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual and relationship[5]. The 10-point improvement in IBS-QOL scoring was clinically meaningful[6]. A lower score indicates a higher quality of life.

Food diary

As diet is a major factor in human gut microbiome variation and function, the dietary profile will need to be recorded for the analysis. Studies assessing direct diet

microbiome relationships have largely relied on food frequency questionnaires (FFQs) and conventional nutrient profiles from macro-and micronutrients[7]. By the way, FFQs in full-length is not practical in use. Therefore, brief dietary instruments can be used instead, which is in the suggestion for using an assessment used in cross-sectional design[8].

Body Mass Index (BMI)

The Body Mass Index (BMI) is a measure of body fat based on height and weight, expressed as kg/m^2. It is commonly used to identify overweight and obesity. In the Asian population, evidence suggests that a BMI of over 23 kg/m^2 indicates overweight, while a BMI of over 25 kg/m^2 indicates obesity and is associated with obesity-related health issues[9, 10]. In addition, gut microbiotas and their functions could be significantly altered in overweight and obese individuals[11-13].

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Supplementary Table 1: Food diary information compared between healthy controls and patients with IBS-C

Food items	Control (n = 16)	IBS-C (n = 16)	*p-value*
Cereals			0.463
Never/rarely	12/16	9/16	
1-3 times/month	3/16	4/16	
1-2 times/week	0/16	2/16	
3-4 times/week	1/16	1/16	
White roots and tubers			0.896
Never/rarely	4/16	4/16	
1-3 times/month	5/16	5/16	
1-2 times/week	6/16	5/16	
3-4 times/week	1/16	1/16	
5-6 times/week	0/16	1/16	
Vitamin A rich vegetables and tubers			0.190
Never/rarely	2/16	1/16	
1-3 times/month	1/16	6/16	
1-2 times/week	8/16	4/16	
3-4 times/week	3/16	2/16	
5-6 times/week	1/16	3/16	
2-3 times/day	1/16	0/16	
Dark green leafy vegetables (DGLV)			0.158
Never/rarely	0/16	1/16	
1-3 times/month	0/16	2/16	
1-2 times/week	3/16	1/16	
3-4 times/week	6/16	2/16	
5-6 times/week	4/16	3/16	
Daily	3/16	4/16	
2-3 times/day	0/16	3/16	
Other vegetables			0.096
Never/rarely	0/16	2/16	
1-3 times/month	0/16	2/16	
1-2 times/week	2/16	6/16	
3-4 times/week	4/16	3/16	
5-6 times/week	5/16	2/16	
Daily	3/16	0/16	
2-3 times/day	2/16	1/16	

Food items	Control (n = 16)	IBS-C (n = 16)	*p-value*
Vitamin A rich fruits			0.570
Never/rarely	4/16	7/16	
1-3 times/month	5/16	5/16	
1-2 times/week	5/16	4/16	
3-4 times/week	1/16	0/16	
Daily	1/16	0/16	
Other fruits			0.401
Never/rarely	2/16	1/16	
1-3 times/month	0/16	2/16	
1-2 times/week	3/16	2/16	
3-4 times/week	7/16	5/16	
5-6 times/week	2/16	2/16	
Daily	2/16	1/16	
2-3 times/day	0/16	3/16	
Flesh foods and organ meat			0.659
Never/rarely	6/16	4/16	
1-3 times/month	5/16	7/16	
1-2 times/week	2/16	1/16	
3-4 times/week	3/16	2/16	
5-6 times/week	0/16	1/16	
2-3 times/day	0/16	1/16	
Eggs			0.279
1-3 times/month	2/16	2/16	
1-2 times/week	2/16	5/16	
3-4 times/week	6/16	2/16	
5-6 times/week	3/16	6/16	
Daily	2/16	0/16	
2-3 times/day	1/16	1/16	
Fish and seafood			0.622
Never/rarely	0/16	2/16	
1-3 times/month	4/16	5/16	
1-2 times/week	6/16	4/16	
3-4 times/week	4/16	4/16	
5-6 times/week	1/16	1/16	
Daily	1/16	0/16	
Bean and peas			0.241
Never/rarely	3/16	4/16	
1-3 times/month	6/16	5/16	
1-2 times/week	5/16	3/16	
3-4 times/week	2/16	0/16	
5-6 times/week	0/16	2/16	

Food items	Control (n = 16)	IBS-C (n = 16)	*p-value*
Daily	0/16	2/16	
Nut and seeds			0.167
Never/rarely	4/16	6/16	
1-3 times/month	7/16	7/16	
1-2 times/week	5/16	1/16	
3-4 times/week	0/16	2/16	
Milk and milk products			0.980
Never/rarely	2/16	2/16	
1-3 times/month	4/16	4/16	
1-2 times/week	2/16	1/16	
3-4 times/week	3/16	4/16	
5-6 times/week	2/16	1/16	
Daily	2/16	2/16	
2-3 times/day	1/16	2/16	
Oil and fats			0.387
1-3 times/month	1/16	2/16	
1-2 times/week	6/16	2/16	
3-4 times/week	5/16	4/16	
5-6 times/week	1/16	4/16	
Daily	3/16	3/16	
2-3 times/day	0/16	1/16	
Sweets			0.187
Never/rarely	4/16	3/16	
1-3 times/month	4/16	2/16	
1-2 times/week	4/16	2/16	
3-4 times/week	2/16	0/16	
5-6 times/week	0/16	3/16	
Daily	2/16	5/16	
2-3 times/day	0/16	1/16	
Spices, condiments, beverages			0.499
Never/rarely	1/16	5/16	
1-3 times/month	2/16	1/16	
1-2 times/week	3/16	2/16	
3-4 times/week	5/16	2/16	
5-6 times/week	2/16	1/16	
Daily	2/16	3/16	
2-3 times/day	1/16	2/16	

Differences between groups were analyzed using the Chi-square (x^2) test.

Supplementary Table 2: Food diary information in patients with IBS-C at pre-treatment, post-treatment, and one-month follow-up

Food items	IBS-C (n = 16)			*p-value*
	Pre-treatment	**Post-treatment**	**One-month follow-up**	
Cereals				0.968
Never/rarely	9/16	8/16	8/16	
1-3 times/month	4/16	4/16	4/16	
1-2 times/week	2/16	3/16	2/16	
3-4 times/week	1/16	1/16	1/16	
Daily	0/16	0/16	1/16	
White roots and tubers				0.798
Never/rarely	4/16	3/16	6/16	
1-3 times/month	5/16	6/16	5/16	
1-2 times/week	5/16	6/16	3/16	
3-4 times/week	1/16	0/16	1/16	
5-6 times/week	1/16	0/16	1/16	
Daily	0/16	1/16	0/16	
Vitamin A rich vegetables and tubers				0.754
Never/rarely	1/16	4/16	2/16	
1-3 times/month	6/16	5/16	5/16	
1-2 times/week	4/16	4/16	6/16	
3-4 times/week	2/16	1/16	2/16	
5-6 times/week	3/16	1/16	1/16	
2-3 times/day	0/16	1/16	0/16	
Dark green leafy vegetables (DGLV)				0.425
Never/rarely	1/16	1/16	2/16	
1-3 times/month	2/16	3/16	0/16	
1-2 times/week	1/16	4/16	3/16	
3-4 times/week	2/16	3/16	4/16	
5-6 times/week	3/16	1/16	3/16	
Daily	4/16	1/16	0/16	
2-3 times/day	3/16	3/16	4/16	

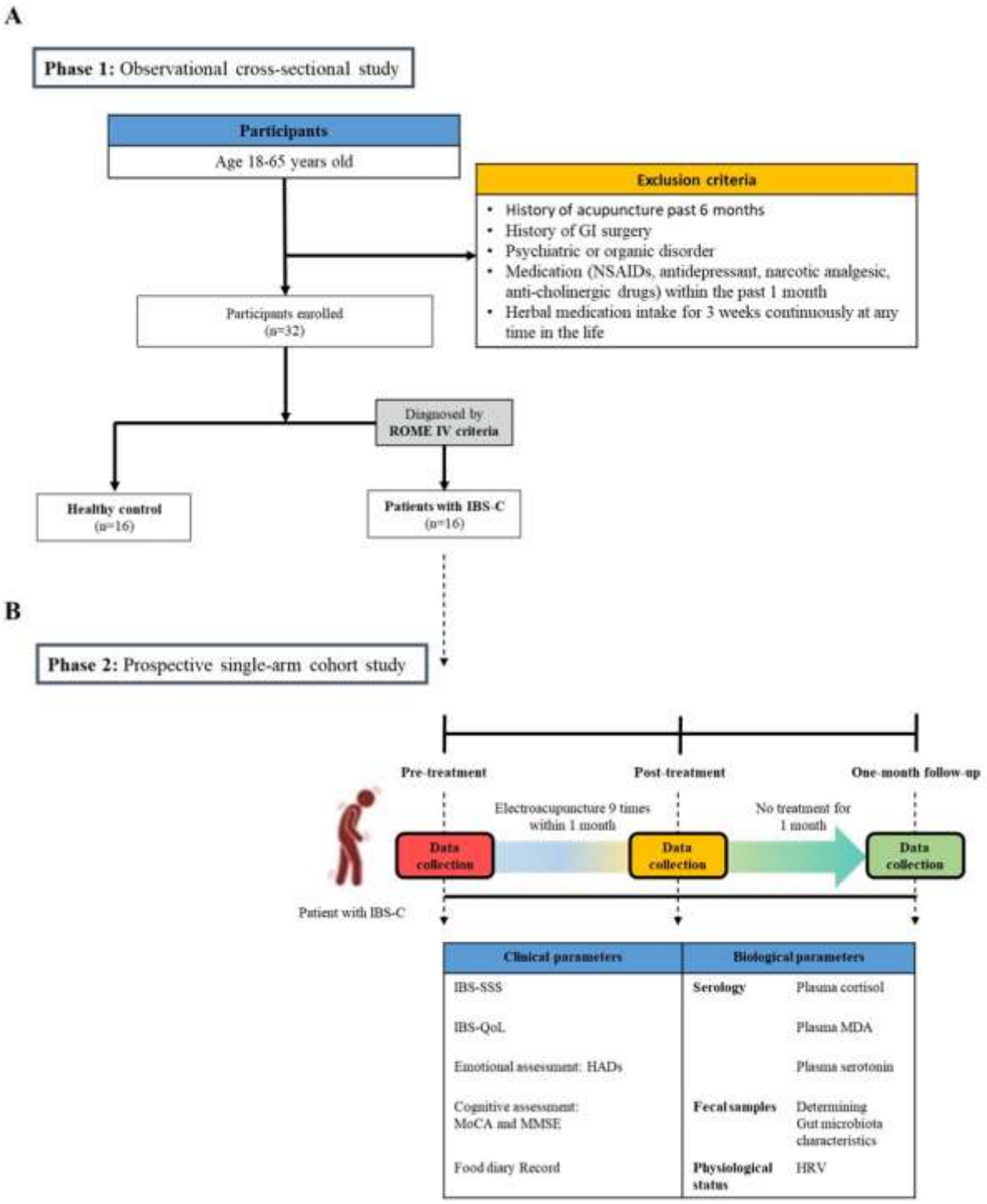
Food items	IBS-C (n = 16)			*p-value*
	Pre-treatment	**Post-treatment**	**One-month follow-up**	
Other vegetables				0.202
Never/rarely	2/16	0/16	1/16	
1-3 times/month	2/16	2/16	1/16	
1-2 times/week	6/16	3/16	7/16	
3-4 times/week	3/16	6/16	1/16	
5-6 times/week	2/16	3/16	2/16	
Daily	0/16	0/16	3/16	
2-3 times/day	1/16	2/16	0/16	
>5 times/day	0/16	0/16	1/16	
Vitamin A rich fruits				0.519
Never/rarely	7/16	10/16	10/16	
1-3 times/month	5/16	4/16	3/16	
1-2 times/week	4/16	1/16	2/16	
3-4 times/week	0/16	0/16	1/16	
Daily	0/16	1/16	0/16	
Other fruits				0.847
Never/rarely	1/16	0/16	0/16	
1-3 times/month	2/16	2/16	2/16	
1-2 times/week	2/16	4/16	6/16	
3-4 times/week	5/16	4/16	4/16	
5-6 times/week	2/16	3/16	1/16	
Daily	1/16	2/16	2/16	
2-3 times/day	3/16	1/16	1/16	
Flesh foods and organ meat				0.579
Never/rarely	4/16	6/16	7/16	
1-3 times/month	7/16	3/16	5/16	
1-2 times/week	1/16	4/16	1/16	
3-4 times/week	2/16	2/16	2/16	
5-6 times/week	1/16	1/16	0/16	
Daily	0/16	0/16	1/16	
2-3 times/day	1/16	0/16	0/16	
Eggs				0.334
Never/rarely	0/16	0/16	1/16	
1-3 times/month	2/16	2/16	1/16	
1-2 times/week	5/16	2/16	5/16	
3-4 times/week	2/16	5/16	3/16	
5-6 times/week	6/16	2/16	2/16	
Daily	0/16	4/16	4/16	

Food items	IBS-C (n = 16)			*p-value*
	Pre-treatment	**Post-treatment**	**One-month follow-up**	
2-3 times/day	1/16	1/16	0/16	
Fish and seafood				0.668
Never/rarely	2/16	0/16	2/16	
1-3 times/month	5/16	4/16	6/16	
1-2 times/week	4/16	8/16	5/16	
3-4 times/week	4/16	3/16	2/16	
5-6 times/week	1/16	1/16	0/16	
Daily	0/16	0/16	1/16	
Bean and peas				0.620
Never/rarely	4/16	7/16	5/16	
1-3 times/month	5/16	4/16	4/16	
1-2 times/week	3/16	2/16	4/16	
3-4 times/week	0/16	1/16	2/16	
5-6 times/week	2/16	0/16	0/16	
Daily	2/16	2/16	1/16	
Nut and seeds				0.054
Never/rarely	6/16	8/16	12/16	
1-3 times/month	7/16	2/16	2/16	
1-2 times/week	1/16	5/16	1/16	
3-4 times/week	2/16	1/16	0/16	
5-6 times/week	0/16	0/16	1/16	
Milk and milk products				0.886
Never/rarely	2/16	2/16	1/16	
1-3 times/month	4/16	2/16	4/16	
1-2 times/week	1/16	1/16	3/16	
3-4 times/week	4/16	6/16	3/16	
5-6 times/week	1/16	1/16	1/16	
Daily	2/16	4/16	3/16	
2-3 times/day	2/16	0/16	1/16	
Oil and fats				0.077
Never/rarely	0/16	0/16	3/16	
1-3 times/month	2/16	1/16	2/16	
1-2 times/week	2/16	8/16	3/16	
3-4 times/week	4/16	4/16	4/16	
5-6 times/week	4/16	0/16	1/16	
Daily	3/16	3/16	1/16	
2-3 times/day	1/16	0/16	2/16	

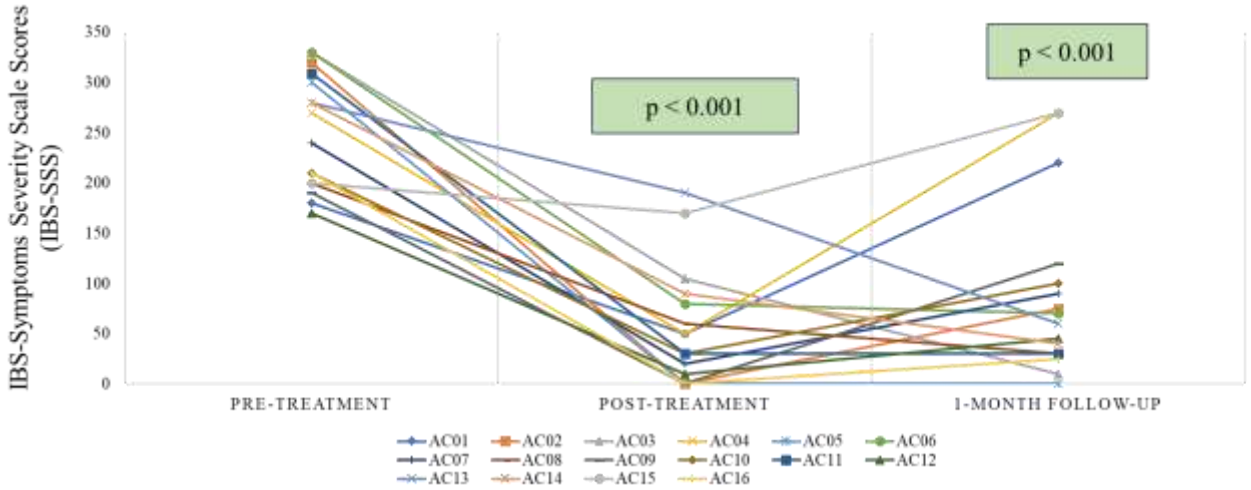
Food items	IBS-C (n = 16)			*p-value*
	Pre-treatment	**Post-treatment**	**One-month follow-up**	
Sweets				0.130
Never/rarely	3/16	3/16	5/16	
1-3 times/month	2/16	1/16	1/16	
1-2 times/week	2/16	4/16	5/16	
3-4 times/week	0/16	5/16	2/16	
5-6 times/week	3/16	0/16	1/16	
Daily	5/16	1/16	1/16	
2-3 times/day	1/16	2/16	0/16	
>5 times/day	0/16	0/16	1/16	
Spices, condiments, beverages				0.882
Never/rarely	5/16	3/16	7/16	
1-3 times/month	1/16	3/16	2/16	
1-2 times/week	2/16	3/16	1/16	
3-4 times/week	2/16	2/16	2/16	
5-6 times/week	1/16	0/16	1/16	
Daily	3/16	3/16	1/16	
2-3 times/day	2/16	2/16	1/16	
>5 times/day	0/16	0/16	1/16	

Differences between groups were analyzed using the Chi-square (x^2) test.

Supplementary Figure 1: The experimental protocol of the study. (A) Phase 1 study. (B) Phase 2 study.



Supplementary Figure 2: The individual trends in IBS-SSS scores across three-time points: pre-treatment, post-treatment, and one-month follow-up.



Supplementary Figure 3: The efficacy evaluation by improvement of IBS-SSS and IBS-QoL scores at post-treatment and one-month follow-up.