Clinic Study Protocol

For article: Decoding a health-related cortical network on chronic fatigue syndrome: Evidence for the reduction of cortical thickness

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Contents

Introduction

This clinic study protocol contains two parts, ① the protocol of the article *Decoding a health-related cortical network on chronic fatigue syndrome: Evidence for the reduction of cortical thickness* and ② the protocol of an entire clinic study protocol.

The part 2 (entire clinic study protocol) is a longitudinal protocol with a treatment of Taichiquan exercise. The part 1 is the protocol designed for data before the treatment of Taichiquan exercise.

The part 1 (protocol of the article) is for the article which focuses on whether patients with chronic fatigue syndrome (CFS) would have cortical thickness reductions compared with healthy volunteers. Part 1 only uses the baseline data of Part 2 for analysis, which means the part 1 belongs to a small portion of part 2 but with a new study objective and analysis design.

Nevertheless, part 1 and part 2 share the same clinic documents, including a registration number ChiCTR2000032577 and a approved number of ethics committee DZMEC-KY-2019-195.

Part 1: The Clinic Study Protocol of the Article (Version Date Feb 14 2023)

1. Research Contents

This study contains two groups, the first group is the patients with chronic fatigue syndrome (CFS) and the second group is the matched healthy volunteers according to sex, and and BMI.

all subjects are scanned by structural magnetic resonance brain imaging (sMRI) and measured by clinic questionnaires including McGill Pain Questionnaire (MPQ), the 36-item short form health survey (SF-36), fatigue Scale-14 (FS-14), etc.

Data analysis: To figure out whether chronic fatigue syndrome would lead to cortices reduction and where the reduction is, the combined methods of voxel-based morphometry (VBM) and multi-voxel pattern analysis (MVPA) are performed to study alterations of surface-based vertex between groups of patients with CFS and healthy volunteers, with taking clinic questionnaires into consideration.

2. Research Protocol

2.1 Research Object

2.1.1 Diagnostic Ceiteria of Modern Medicine

Refer to the diagnostic criteria of CFS formulated by CDC in 1994, including:

a. ① chronic fatigue cannot be explained by clinic and have been occurred continuously or intermittently more than 6 months. ② chronic fatigue was not the results of long-term exertion and cannot be relieved obviously after a sufficient rest. ③ chronic fatigue leads to a great impacts to work and daily life.

b. The patient with CFS has at least four of the following eight symptoms that present at the same time, and the occurrence of these symptoms would be more than half a year but later than fatigue:

① significant memory loss or inability to concentrate. ② pharyngalgia. ③ swelling and tenderness of cervical or axillary lymph nodes. ④ muscle pain. ⑤ multiple joint pain without redness and swelling. ⑥ a new type of severe headache. ⑦ sleep disorders. ⑧ fatigue after exercise for more than 24 hours.

2.1.2 Diagnostic Criteria of Traditional Chinese Medicine

Not applicable.

2.1.3 Included Criteria

① meet CFS diagnostic criteria. ② age 25-65 years old, male or female, right-handed. ③ the course of disease is more than six months. ④ chronic fatigue cannot be explained by current diseases. ⑤ there is no history of Taichiquan practice. ⑥ can normally answer and fill questionnaires, with good compliance. ⑦ haven't taken psychotropic drugs in the past month. ⑧ no metal in the body and no contraindication of nuclear magnetic examination. ⑨ sign informed consent.

Note: patients who do not meet any of the above conditions will not be included.

2.1.4 Excluded Criteria

① chronic fatigue caused by the disease directly, such as hypothyroidism, patients with hepatitis B or C virus infection. ② chronic fatigue caused by drug side effects. ③ having been previous diagnosed with emotional disorders and various mental disorders, such as paranoia, anorexia nervosa, etc. ④ suffering from serious heeart, kidney, lung, liver, brain and other major diseases, serious liver and kidney dysfunction. ⑤ severe obesity, body mass index = $[weight(kg)/heght^2(m)] > 45$. ⑥ patients taking vasodilators in recent two weeks. ⑦ pregnant, lactating and girls who had menstruation during sMRI scanning. ⑧ unable to understand and cooperate with the examination or other

MRI contraindications such as claustrophobia. ⑨ severe asymmetry of skull anatomical structure or definite lesions were found in MRI scan. ⑩ those who have participated in similar neuro-imaging experiments within one month.

Note: the subject will be excluded if any of the above conditions is met.

2.1.5 Exit Criteria

① subjects wanted to quit. ② subjects failed to complete the information recording as required that effects results evaluation. ③ subjects missed. ④ subjects failed to carry out Taichiquan training as required.

2.1.6 Suspension Criteria

① subjects with poor compliance and failed to perform MRI examination and safety evaluation as required shall be excluded. ② cases with serious adverse events or complications that are not suitable to continue and the trial is suspended.

2.1.7 Reject Criteria

All subjects that do not meet the inclusion criteria and are entered by mistake should be excluded.

2.2 Sample Size Evaluation

In the field of Chronic Fatigue Syndrome (CFS) neuroimaging research, challenges persist due to a generally small sample size, recurrent CFS attacks, and occasional issues with patient compliance. The range of sample sizes varies, with a minimum of one case and a maximum of fifty cases, most of which cluster between 20 and 40 cases. Considering factors such as subject demographics, statistical requirements, and other relevant considerations, it is recommended to maintain a sample size of at least 40 for both CFS patients and healthy subjects.

2.3 Study Group

This study contains two groups, one is the patients group with subjects who were diagnosed with CFS, the second is the healthy control group with matched-volunteers according to sex, age and BMI of patients group. This experiment belongs to a small case control study without randomization.

2.4 Treatment Plan

Not applicable. No treatment.

2.5 Observation Indicators and Time Points

2.5.1 Outcome Indicators

The primary outcome measure: the MOS item short from health survey (SF-36).

The secondary outcome measure: the McGill Pain Questionnaire (MPQ) and Fatigue Scale-14 (FS-14).

2.5.2 Safety Indicators

Blood pressure, heart rate, respiration, etc.

2.6 Efficacy Evaluation Criteria

The efficacy evaluation standard of this study takes the improvement of SF-36 scale after statistical analysis as the main standard, and the statistical difference of MPQ and FS-14 as the secondary standard.

2.7 Adverse Event

In the event that subjects experience discomfort during MRI scanning, skilled medical professionals will promptly diagnose and administer necessary treatments.

2.8 Data Record and Statistical Analysis

2.8.1 Data Record

a. The research group has developed a comprehensive researcher's operations manual to precisely outline the collection of information and data. Additionally, the group conducts clinical research training for researchers, aiming to guarantee the accuracy, integrity, and consistency of the gathered information and data.

b. Following the specifications outlined in the research plan, the research team will create a standardized case report form and implement structured processing for clinically collected information. This approach aims to uphold the objectivity and consistency of the information collected throughout the study.

c. The researcher is responsible for ensuring that data is accurately, completely, clearly, and promptly entered into the case report form in accordance with the original observations of the subjects.

2.8.2 sMRI Data Acquisition

2.8.2.1 Scanning Parameters After locating the structural image in the conventional three planes, axial scanning of the structural image is conducted using the T1-weighted fast phase disturbing gradient echo sequence (FSPGR). Weighted positioning is performed based on the vertical line of the connecting line between the anterior union and the posterior union, encompassing the entire brain region from the skull top to the skull base. The scanning parameters include: TR (repetition time) / TE (echo time) = $2530\ ms/3.4\ ms$, FOV (field of view) = $240\ mm \times 240\ mm$, matrix = 512×512, turning angle: 12°, and layer thickness: $1\ mm$.

2.8.2.2 Scanning Protocol The identical scan sequence was employed for both MRI scans. The scanned individual rested in the supine position for a duration of 30 minutes. Following complete relaxation, maintaining an audio-visual closed state, securing the magnetic resonance coil, stabilizing the head, and the T1 structural image was scanned for 4 minutes and 10 seconds.

2.8.3 Statistical Analysis

2.8.3.1 General Clinical Data Statistics Statistical analysis for this experimental study will be conducted using R software. The statistical significance test will be a two-sided test, with a significance level set at $P < 0.05$. Quantitative data will be analyzed using means, standard deviation, the number of cases, minimum and maximum values, 95 confidence interval. Inter-group comparison will be performed as needed.

For inter-group comparisons, the t-test for two independent samples will be employed to check the comparability of basic information between two groups if the data in both groups follow a normal distribution with homogeneous variances; otherwise, the Wilcoxon rank sum test will be used.

2.8.3.2 Statistical Analysis of sMRI Data

a. Get VMB data: firstly, the originally structural DICOM images are converted to bids format and processed by DPABISurf software, which is an ensemble MRI analysis tool based on fMRIPrep and Freesurfer. Specifically, by applying the fMRIPrep framework, T1-weighted images from each subject are corrected for intensity, non-uniformity and skull-stripped. Then, the stripped image is segmented to gray matter, white matter, and cerebrospinal fluid. Next, the recon-all command is used to reconstruct the brain surface of the subject in Freesurfer software, and the former segmented gray matter is normalized to standard MNI152 volume space by the nonlinear registration method using the MNI152NLin2009cAsym template. Finally, using DPABISurf software, the voxel size of the gray matter on standard volume space is resampled to $2 \times 2 \times 2\ mm^3$. Thus, the prepared gray matter data of voxel-based morphometry (VBM) are prepared for further VBM decoding.

b. Transfer VBM data to surface space: in addition, when the recon-all command is executed, the surface thicknesses of the left and right hemispheres of each subject are generated in Fsaverage surface space. Then, DPABISurf 1.6 software is used to smooth the thicknesses with a Gaussian kernel of 6 mm^3. Hence, the surface thickness data are prepared for further comparison.

c. Decode surface thickness: Nilearn package is used to perform multi-voxel pattern analysis (MVPA). A random seed is first set for the whole process to help reproducibility. After standardization of the VBM matrix, ratio 0.5 is used to randomly divide the matrix into a training dataset and a test dataset, with 35 subjects in the training set and 36 subjects in the test dataset. Further, a support vector classifier (SVC) model is applied. Then, half-split cross validation and 5,000 times half-split permutation test are used to measure model performances.

2.9 Quality Control

2.9.1 Quality control of sMRI scanning

a. Prior to MRI scanning, the individual undergoing the scan is provided with detailed information regarding the scanning time, purpose, and relevant precautions. Following the individual's adaptation to the indoor environment, they are instructed to rest in a supine position for a duration of 30 minutes. Scanning commences once the subjects has achieved a state of complete calmness.

b. Safety index monitoring is conducted each scan to assess and evaluate the safety of the testing process.

c. Building upon insights from prior studies, enhancements to the Case Report Form (CRF) have been implemented. The revised CRF now comprehensively records the patient's basic information, detailed condition, and the alterations in vital signs for each scan. Additionally, any adverse events arising during the research process are promptly documented.

Part 2: The Entire Clinic Study Protocol Design (Version Date Dec 28 2019)

1. Research Contents

This study contains two groups, the first group is the patients with chronic fatigue syndrome (CFS) and the second group is the matched healthy volunteers according to sex, and and BMI. The subjects of two groups both receive 24 style Taichiquan training for four weeks. Before and after Taichiquan training, all subjects are scanned by magnetic resonance brain imaging (MRI) and measured by clinic questionnaires including McGill Pain Questionnaire (MPQ), Pittsburgh sleep quality index (PSQI), Hamilton Depression Scale (HAMD), health survey brief (SF-36), fatigue Scale-14 (FS-14), etc.

Data analysis has two parts. (1) Baseline period: ① compare the sociodemographic characters and scores of clinic questionnaires between patients with chronic fatigue syndrome and healthy volunteers to evaluate the disease severity of patients with CFS, and ② compare the differences between MRI data of two groups to explore alterations of brain function in patients with CFS. (2) After Taichiquan training: compare changes of scores of clinic questionnaire for two groups at levels of within-group and between-group.

2. Research Protocol

2.1 Research Object

2.1.1 Diagnostic Ceiteria of Modern Medicine

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a. ① chronic fatigue cannot be explained by clinic and have been occurred continuously or intermittently more than 6 months. ② chronic fatigue was not the results of long-term exertion and cannot be relieved obviously after a sufficient rest. ③ chronic fatigue leads to a great impacts to work and daily life.

b. The patient with CFS has at least four of the following eight symptoms that present at the same time, and the occurrence of these symptoms would be more than half a year but later than fatigue:

① significant memory loss or inability to concentrate. ② pharyngalgia. ③ swelling and tenderness of cervical or axillary lymph nodes. ④ muscle pain. ⑤ multiple joint pain without redness and swelling. ⑥ a new type of severe headache. ⑦ sleep disorders. ⑧ fatigue after exercise for more than 24 hours.

2.1.2 Diagnostic Criteria of Traditional Chinese Medicine

Not applicable.

2.1.3 Included Criteria

① meet CFS diagnostic criteria. ② age 25-65 years old, male or female, right-handed. ③ the course of disease is more than 6 months. ④ chronic fatigue cannot be explained by current diseases. ⑤ there is no history of Taichiquan practice. ⑥ can normally answer and fill questionnaires, with good compliance. ⑦ haven't taken psychotropic drugs in the past month. ⑧ no metal in the body and no contraindication of nuclear magnetic examination. ⑨ sign informed consent.

Note: patients who do not meet any of the above conditions will not be included.

2.1.4 Excluded Criteria

① chronic fatigue caused by the disease directly, such as hypothyroidism, patients with hepatitis B or C virus infection. ② chronic fatigue caused by drug side effects. ③ having been previous diagnosed with emotional disorders and various mental disorders, such as paranoia, anorexia nervosa, etc. ④ suffering from serious heeart, kidney, lung, liver, brain and other major diseases, serious liver and kidney dysfunction. ⑤ severe obesity, body mass index = $[weight(kg)/height^2(m)] > 45$. ⑥ patients taking vasodilators in recent two weeks. ⑦ pregnant, lactating and girls

who had menstruation during MRI scanning. ⑧ unable to understand and cooperate with the examination or other MRI contraindications such as claustrophobia. ⑨ severe asymmetry of skull anatomical structure or definite lesions were found in MRI scan. ⑩ those who have participated in similar neuro-imaging experiments within one month.

Note: the subject will be excluded if any of the above conditions is met.

2.1.5 Exit Criteria

① subjects wanted to quit. ② subjects failed to complete the information recording as required that effects results evaluation. ③ subjects missed. ④ subjects failed to carry out Taichiquan training as required.

2.1.6 Suspension Criteria

① subjects with poor compliance and failed to perform MRI examination and safety evaluation as required shall be excluded. ② cases with serious adverse events or complications that are not suitable to continue and the trial is suspended.

2.1.7 Reject Criteria

All subjects that do not meet the inclusion criteria and are entered by mistake should be excluded.

2.2 Sample Size Evaluation

In the field of Chronic Fatigue Syndrome (CFS) neuroimaging research, challenges persist due to a generally small sample size, recurrent CFS attacks, and occasional issues with patient compliance. The range of sample sizes varies, with a minimum of one case and a maximum of fifty cases, most of which cluster between 20 and 40 cases. Considering factors such as subject demographics, statistical requirements, and other relevant considerations, it is recommended to maintain a sample size of at least 40 for both CFS patients and healthy subjects.

2.3 Study Group

This study contains two groups, one is the patients group with subjects who were diagnosed with CFS, the second is the healthy control group with matched-volunteers according to sex, age and BMI of patients group. This experiment belongs to a small case control study without randomization.

2.4 Treatment Plan

To minimize the impact of training time advantages, the frequency and duration of Taichiquan sessions for both the CFS group and the healthy control group have been standardized. Both groups undergo training twice a week, with each session lasting 30 minutes. The training sessions, spanning a 4-week course with a total of 8 sessions, focus on movement learning and repetitive posture control under therapist guidance. In addition to the supervised sessions, participants are instructed to practice Taichiquan at home for 30 minutes daily during the rest of the week.

The standard movements for Taichiquan rehabilitation training adhere to the guidelines outlined in the simplified 24 Style Taichiquan, as endorsed by the General Administration of Sports of the People's Republic of China. Additionally, the prescribed Taichiquan movements are drawn from the standard repertoire detailed in the textbook "Chinese Traditional Health Care: Sports and Health Preservation," widely recognized in national colleges and universities.

2.5 Observation Indicators and Time Points

2.5.1 Outcome Indicators

The primary outcome measure: the MOS item short from health survey (SF-36).

The secondary outcome measure: McGill Pain Questionnaire (MPQ), Pittsburgh Sleep Quality Index (PSQI), Hamilton Depression Scale (HAMD), Fatigue Scale-14 (FS-14), etc.

2.5.2 Safety Indicators

Blood pressure, heart rate, respiration, etc.

2.6 Efficacy Evaluation Criteria

The efficacy evaluation standard of this study takes the improvement of SF-36 scale after statistical analysis as the main standard, and the statistical difference of MPQ, PSQI, HAMD and FS-14 as the secondary standard. Resting-state MRI scanning is the research background of the mechanism.

2.7 Adverse Event

In the event that subjects experience discomfort during MRI scanning, skilled medical professionals will promptly diagnose and administer necessary treatments. The selection of Taijiquan coaches for instruction will be entrusted to experienced professionals, chosen by the researcher from the rehabilitation department, which possesses specific expertise in sports injury rehabilitation. In cases where participants encounter sports injuries throughout the project, the rehabilitation teacher within the project group is equipped to provide tailored rehabilitation training.

2.8 Data Record and Statistical Analysis

2.8.1 Data Record

a. The research group has developed a comprehensive researcher's operations manual to precisely outline the collection of information and data. Additionally, the group conducts clinical research training for researchers, aiming to guarantee the accuracy, integrity, and consistency of the gathered information and data.

b. Following the specifications outlined in the research plan, the research team will create a standardized case report form and implement structured processing for clinically collected information. This approach aims to uphold the objectivity and consistency of the information collected throughout the study.

c. The researcher is responsible for ensuring that data is accurately, completely, clearly, and promptly entered into the case report form in accordance with the original observations of the subjects.

2.8.2 MRI Data Acquisition

2.8.2.1 Scanning Parameters

a. After locating the structural image in the conventional three planes, axial scanning of the structural image is conducted using the T1-weighted fast phase disturbing gradient echo sequence (FSPGR). Weighted positioning is performed based on the vertical line of the connecting line between the anterior union and the posterior union, encompassing the entire brain region from the skull top to the skull base. The scanning parameters include: TR (repetition time) / TE (echo time) = $2530\ ms/3.4\ ms$, FOV (field of view) = $240\ mm \times 240\ mm$, matrix = 512×512, turning angle: $12°$, and layer thickness: $1\ mm$.

b. The functional imaging employs the single-shot plane echo gradient echo sequence (GRE-EPI sequence). The scanning parameters for this include: TR (repetition time) / TE (echo time) = $2000\ ms/30\ ms$, matrix = 64×64, FOV (field of view) = $240\ mm \times 240\ mm$, turning angle $90°$, slice thickness $5\ mm$, continuous cross-sectional scanning of the whole brain without septum, voxel size = $3.75\ mm \times 3.75\ mm \times 5\ mm$.

2.8.2.2 Scanning Protocol The identical scan sequence was employed for both MRI scans. The scanned individual rested in the supine position for a duration of 30 minutes. Following complete relaxation, maintaining an audio-visual closed state, securing the magnetic resonance coil, stabilizing the head, and ensuring continuous scanning in the resting state for 8 minutes. Subsequent to the resting state scan, the T1 structural image was scanned for 4 minutes and 10 seconds, followed by the DTI image scan lasting 5 minutes and 10 seconds.

2.8.3 Statistical Analysis

2.8.3.1 General Clinical Data Statistics Statistical analysis for this experimental study will be conducted using SPSS 20.0 software. The statistical significance test will be a two-sided test, with a significance level set at $P < 0.05$. Quantitative data will be analyzed using means, standard deviation, the number of cases, minimum and maximum values, 95 confidence interval, or median, upper quartile (Q3), and lower quartile (Q1). Inter-group or intra-group comparisons will be performed as needed.

For inter-group comparisons, the t-test for two independent samples will be employed if the data in both groups follow a normal distribution with homogeneous variances; otherwise, the Wilcoxon rank sum test will be used. In the case of intra-group comparisons, the paired t-test will be used if the differences between before and after conform to a normal distribution; otherwise, the signed rank sum test will be applied. Covariance analysis will be used if other factors are considered.

Qualitative data will undergo descriptive statistical analysis, including frequency and composition ratio. Group and within-group comparisons will be conducted as necessary, utilizing the $\chi 2$ test or Fisher exact probability method for comparisons between groups, and the Wilcoxon rank sum test for ordinal data. Chi-square test will be used for intra-group comparisons. The CMH chi-square test will be applied if the center effect is considered.

2.8.3.2 Statistical Analysis of MRI Data Firstly, the structural image is aligned uniformly to the AC-PC (anterior commissioner-posterior commissioner) line using custom code. Following this, cortex segmentation is performed, separating the scalp and sub-cortical regions, and distinguishing various cortical partitions. The result is the acquisition of a cortical structure image. Subsequently, this cortical structure image is standardized to the standard coordinates on the AFNI platform, resulting in the generation of the reprocessed image.

The cortical structure image, standardized to the standard coordinates, is aligned with the EPI image using the FreeSurfer software package, yielding an alignment matrix. This matrix is subsequently projected and applied to the EPI image, resulting in an EPI image aligned with the cortical structure image. To enhance the data, the obtained EPI image undergoes Gaussian smoothing through the SPM8 software package.

Independent component analysis is applied to the Gaussian smoothed image, and the number of components is determined using the Minimum Description Length (MDL) criterion within the GIFT software. Subsequently, the ICASSO method is employed to sift through the components, utilizing power calculation and the best fit method to further narrow down the selection. The final components of interest are then chosen. Using the SPM8 software package, a generalized linear model is employed to analyze these selected components, revealing differences in related brain networks across different states.

The investigation into the relationship between brain networks primarily involves conducting multivariate Granger analysis. This analysis is applied to the different components obtained, aiming to preliminary explore the relationship and fundamental time dynamics between these brain networks.

The initial DTI image undergoes correction for eddy current, followed by the utilization of the corrected image for diffusion tensor computation. The diffusion tensor for each subject undergoes both linear and nonlinear alignment with the internationally accepted diffusion tensor template. The characteristics of all subjects are aggregated to create a standardized diffusion tensor template. This template is then employed to generate a fractional anisotropy (FA) skeleton, facilitating the computation of FA values for each subject. Ultimately, the FSL software package is employed for inter-group comparisons.

2.9 Quality Control

2.9.1 Quality control of Taichiquan exercise

To ensure subject homogeneity, prior to the commencement of the study, all therapists received training from a Taichiquan instructor who had won the second prize in the national Taichiquan Wushu competition. This training aimed to standardize the essential movements of Taichiquan across all therapists involved in the study.

2.9.2 Quality control of MRI scanning

a. Prior to MRI scanning, the individual undergoing the scan is provided with detailed information regarding the scanning time, purpose, and relevant precautions. Following the individual's adaptation to the indoor environment, they are instructed to rest in a supine position for a duration of 30 minutes. Scanning commences once the patient has achieved a state of complete calmness.

b. Safety index monitoring is conducted both before and after each scan to assess and evaluate the safety of the testing process.

c. Building upon insights from prior studies, enhancements to the Case Report Form (CRF) have been implemented. The revised CRF now comprehensively records the patient's basic information, detailed condition, and the alterations in vital signs both before and after each scan. Additionally, any adverse events arising during the research process are promptly documented. Furthermore, the emergency treatment plan has been refined to ensure an improved and more robust response to unforeseen events.