Study protocol and statistical analysis of

Bio-significance of LPC16:0 in Fibromyalgia

Identifiers: NCT04832100

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This observational study is prospectively approved by the Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-(I)-20170012) and registered at ClinicalTrials.gov (identifier NCT04832100; date of registration: April 5, 2021). Adult patients with FM are consecutively enrolled from the outpatient department since June 1, 2021. All participants are well informed and provided written informed consent. Potential participants are interviewed by neurologists, and those who met the 2011 American College of Rheumatology criteria for FM are included. To assess symptoms before pharmacotherapy and track the treatment responses, only patients with a new diagnosis of primary FM not receiving pharmacotherapy are included for analysis. None of the participants had received treatment with anti-epileptic agents, serotonin norepinephrine reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs) or tricyclic antidepressants within the 3 months preceding the study. Age- and sex-matched healthy individuals are prospectively enrolled as healthy controls (HCs) from the outpatient department and public recruitment. Exclusion criteria for both patient and control groups included the presence of a systemic immune disorder, a known history of psychiatric or personality disorders, chronic pain disorders other than FM (e.g., diabetes neuropathic pain, neuralgia, etc.), malignancy, systemic use of corticosteroids, pregnancy, a major systemic disorder (e.g., kidney/liver dysfunction) and a chronic disease under poor control (e.g., diabetes mellitus or hypertension). We also excluded participants who might not be able to provide unbiased clinical information, such as those involved in ongoing litigation or filing compensation.

Study design

The clinical data for patients and controls are obtained through questionnaires and interviews with the same pain specialists (KWL, YOF and CHH) throughout the study. Pain intensity of individuals is assessed with numeric rating scale for pain (NRS) and pain diffuseness by widespread pain index (WPI). The states of anxiety and depression are assessed using the Hospital Anxiety and Depression Scale (HADS). We considered the changes in FM severity as the outcomes of interest, which are assessed using the Revised Fibromyalgia Impact Questionnaire (FIQR)

9. To record the initial therapeutic responses, all patients are under a follow-up procedure for at least one month after treatment. Cases with missing values or incomplete follow-up are excluded from analysis. All patients completed the questionnaires during the initial interview, and follow-up assessments are scheduled at 2 and 4 weeks to monitor their therapeutic responses. During the first visit, comprehensive interviews are performed. We initially educated patients about nonpharmacological therapies, such as exercise and relaxation techniques, and emphasize their importance throughout the treatment process. In this non-trial observational study, all participants received adequate pharmacotherapy in accordance with existing therapeutic guidelines. All participants agreed to receive pharmacotherapy, and no placebo group is included. To target pain control and minimize interference with depressive symptoms, we used pregabalin (75 mg, twice a day) as initial pharmacotherapy, with or without a low dose of imipramine (25 mg, once a day). Participants who are intolerant to pregabalin or imipramine are excluded from analyses. No SNRIs, SSRIs, morphine or opioid-analogue drug is used for pain control during the initial 4 weeks of treatment. After 4 weeks, by clinical judgment, patients with sustained or deteriorated emotional symptoms are referred to a psychiatrist for further management, including cognitive behavioral therapy and antidepressants.

Statistical analysis

Data are expressed as mean ± standard deviation. Sample size calculations are performed using the statistical software G* Power (version 3.1.9.2), with a 0.05 significance level ($\alpha = 0.05$) and a power of 80% ($\beta = 0.20$) in all calculations. An effect size is determined according to previous literature. For a comparison of two groups, a sample size of 45 per group would have 80% power to detect an effect size of 0.6 using a t-test with a 5% two-sided significance level. For regression analysis, an optimal sample size is at least 26 for an effect size of 0.6 and four predictors (NRS, WPI, HADS-A and HADS-D scores).

Between-group differences are assessed using the Student's t-test; Levene’s test is performed beforehand to confirm the homogeneity of variances between groups. The longitudinal analysis of changes after treatment are compared using

repeated measures ANOVA. Pearson correlation is used to assess the correlation between parameters. Cluster analysis is performed to identify distinct groups based on anxiety and depression severity. The k-means clustering algorithm is used with the HADS-Anxiety (HADS-A) and HADS-depression (HADS-D) scores, and patients with similar manifestations are grouped into 2 subgroups. Repeated measures correlation (rmcorr) is used to determine the within-individual associations of clinical symptoms and disease severity, with repeated assessments across multiple individuals. Multiple linear regression (MLR) analysis is performed to determine the clinical factors associated with disease improvement after treatment. The linear mixed-effect regression model (LMM) is used to investigate the influences of symptomatic factors on FM severity during follow-up. Mediation analyses are conducted to assess the mediation effects of pain and mood symptoms on disease severity. Detailed information for correlation and mediation analyses is described in the Supporting Information. Two-tailed $p < 0.05$ is considered statistically significant. For the analyses of multiple tests, such as correlation analyses and ANOVA, the resulting p values are adjusted through Bonferroni correction, with $p < 0.0125$ considered statistically significant. SPSS v20 (SPSS, USA) with the PROCESS macro used for analysis.