

Randomized trial, parallel-group to compare effect of low-intensity transcranial magnetic stimulation (Li-TMS) on sleep disorders, inflammation levels, and brain-derived neurotrophic factor in patients with depression.

NCT ID not yet assigned. 05/12/2024.

Introduction

Depression is a major global mental health barrier and the leading cause of mental disorder disability worldwide. It is a common condition that often begins early in life. According to data published by the World Health Organization, it is estimated that around 4% of the population suffers from depression, including 5.7% of adults (4.6% men and 6.9% women) and 5.9% of adults aged 70 and over (1).

Globally, the coronavirus disease 2019 (COVID-19) pandemic significantly increased the incidence and prevalence of depression. An estimated 53.2 million additional cases of major depressive disorder (MDD) were reported, representing an approximate increase of 27.6% (2).

In Mexico, the National Health and Nutrition Survey (ENSANUT, 2023) reports a prevalence of 13,671 cases per 100,000 inhabitants, equivalent to 16.1% of the adult population nationwide. This situation has contributed to suicide becoming the fourth leading cause of death among people aged 15 to 29 (3).

In low- and middle-income countries, only 25% of patients diagnosed with depression receive some form of treatment, with the current options being standard drug therapy and/or psychotherapy (WHO, 2023) (1). However, only about 35% of patients achieve complete remission of symptoms, resulting in more than half of patients with treatment-resistant depression (4).

Several studies have evaluated the efficacy of bilateral high-frequency (10 Hz) (5,6) and low-frequency (1 Hz) (50) Li-TMS applied to the dorsolateral prefrontal cortex (DLPFC) in patients with depression and insomnia over a 6-week period, totaling 30 sessions. These studies showed improvement in depressive symptoms and sleep quality in adults beginning at session 10 (5) and 15, respectively, as assessed by the Pittsburgh Sleep Quality Index (PSQI) and the Patient Health Questionnaire-9 (PHQ9) (7).

The exact mechanism by which Li-TMS improves sleep quality in patients with depression remains unclear. However, evidence indicates that patients who underwent high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) demonstrate improvement in sleep patterns, improvement in sleep stage, positive correlations in motor evoked potential, negative correlations with GABA and BDNF levels, as well as decreased levels of thyroid hormone, adrenocorticotrophic hormone, and thyroid-stimulating hormone (5).

In 2013, Saeki et al. analyzed the effects of HF-rTMS on sleep structure in patients with depression. This study included 12 male patients with moderate treatment-resistant depression who underwent a total of 10 HF-rTMS sessions in the DLPFC. Polysomnographic studies were performed (adaptation, baseline, Pot1 after 5 sessions, and post 2 after 10 sessions). They demonstrated that high-frequency r-TMS (20 Hz) in the DLPDFC induces an increase in Delta power band F3 (left upper lobe) of the brain during sleep after 5 sessions, improving depression and daytime sleepiness, and an increase in N2 to N3 sleep stages in these patients. However, no changes were found in the last 5 sessions of the study, suggesting that homeostatic control of the sleep cycle can be restored with an improvement in slow wave activity. Nevertheless, this study has

multiple limitations, and further studies are needed to evaluate the effect of rTMS on sleep architecture through polysomnography studies (7, 8).

In recent years, evidence has accumulated linking immune alterations to mood disorders, highlighting the role of the inflammatory response system (IRS) (33-36). Activation of the IRS with overproduction of inflammation-regulating cytokines affects various mechanisms associated with mood, cognition, and emotional regulation, including neurotransmission, microglial activation, dysregulation of the hypothalamic-pituitary-adrenal axis, and brain plasticity (9-11).

A meta-analysis on major depressive disorder confirmed higher mean levels of C-reactive protein (CRP) in patients with MDD compared to controls (12) with moderate heterogeneity ($Q = 51$, $p < 0.0001$, $I^2 = 62\%$). In patients with first-episode (MDD) who had not received pharmacological treatment (13), a meta-analysis of five studies involving 179 patients and 210 healthy controls (HC) found significantly higher CRP levels in the MDD group ($g = 0.53$; 95% CI = 0.24-0.82; $p < 0.001$). Interestingly, a recent study found a positive correlation between circulating CRP levels, cerebrospinal fluid (CSF) concentrations of CRP and cytokine receptors/antagonists and the severity of depressive symptoms in patients with the highest CRP levels (14).

Recent studies have shown that inflammatory hematological indices such as the neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are elevated in patients with major depression compared to control groups (SMD = 0.33, 95% CI: 0.15–0.52, $p < 0.001$ and SMD = 0.24, 95% CI: 0.02–0.46, $p < 0.05$, respectively). Hu et al. report a mean PRL of 120.19 ± 44.15 in healthy controls and a mean PRL of $195.25 \pm$

112.26 in patients with depression, suggesting its possible usefulness as an accessible biomarker of the inflammatory state associated with MDD (15-18).

On the other hand, BDNF expression and signaling are important for understanding the underlying mechanisms of MDD. BDNF has been observed to act as a mediator of synaptic plasticity, suggesting that a decrease in this factor correlates with reduced synaptic plasticity and neuronal atrophy, which could be the basis of depression (19). It has been shown that chronic conventional antidepressant treatments tend to increase BDNF expression in the cortex and hippocampus, promoting neuronal recovery and clinical improvement (20).

In 2023, Yoshimura et al. compared changes in serum brain-derived neurotrophic factor (BDNF) concentrations among patients with first-episode major depressive disorder (MDD), comparing those who demonstrated a therapeutic response to antidepressant treatment with those who did not. Their results showed that serum BDNF levels were not different in either groups. however, patients who responded to treatment showed significant changes in serum BDNF (10.2 vs. 11.1 ng/mL), i.e., serum BDNF increased after 8 weeks of treatment (21).

Reduced serum BDNF concentrations have been observed in patients with depression who have not received pharmacological treatment. Following exposure to antidepressant therapy, these patients typically exhibit increased serum BDNF levels accompanied by a reduction in Hamilton Depression Rating Scale (HAM-D) scores. BDNF were lower in patients with MDD who had not received prior treatment compared to healthy individuals (0.3878 vs. 0.72377 ng/mL) (22). An association has also been demonstrated between low serum BDNF levels in patients with MDD and suicidal ideation compared to patients with

MDD without suicidal ideation (z-score -0.26 vs. 0.34) (23). Therefore, it is advisable to integrate the relationship between BDNF levels and MDD into studies on complementary treatments such as TMS.

Sample size calculation

Based on the results of Holczer et al., the depression symptom score was reduced by 50% (8.2 ± 3.36 vs. 4.2 ± 1.17). (32) The sample size calculation is based on a 95% confidence level, a 5% margin of error, and a standard deviation of 3.36 obtained from the 2021 study by Holczer et al. (32), resulting in a total of 11.67, rounded to 40 to account for sample loss, so each group would consist of 20 participants, for a total of 40 patients.

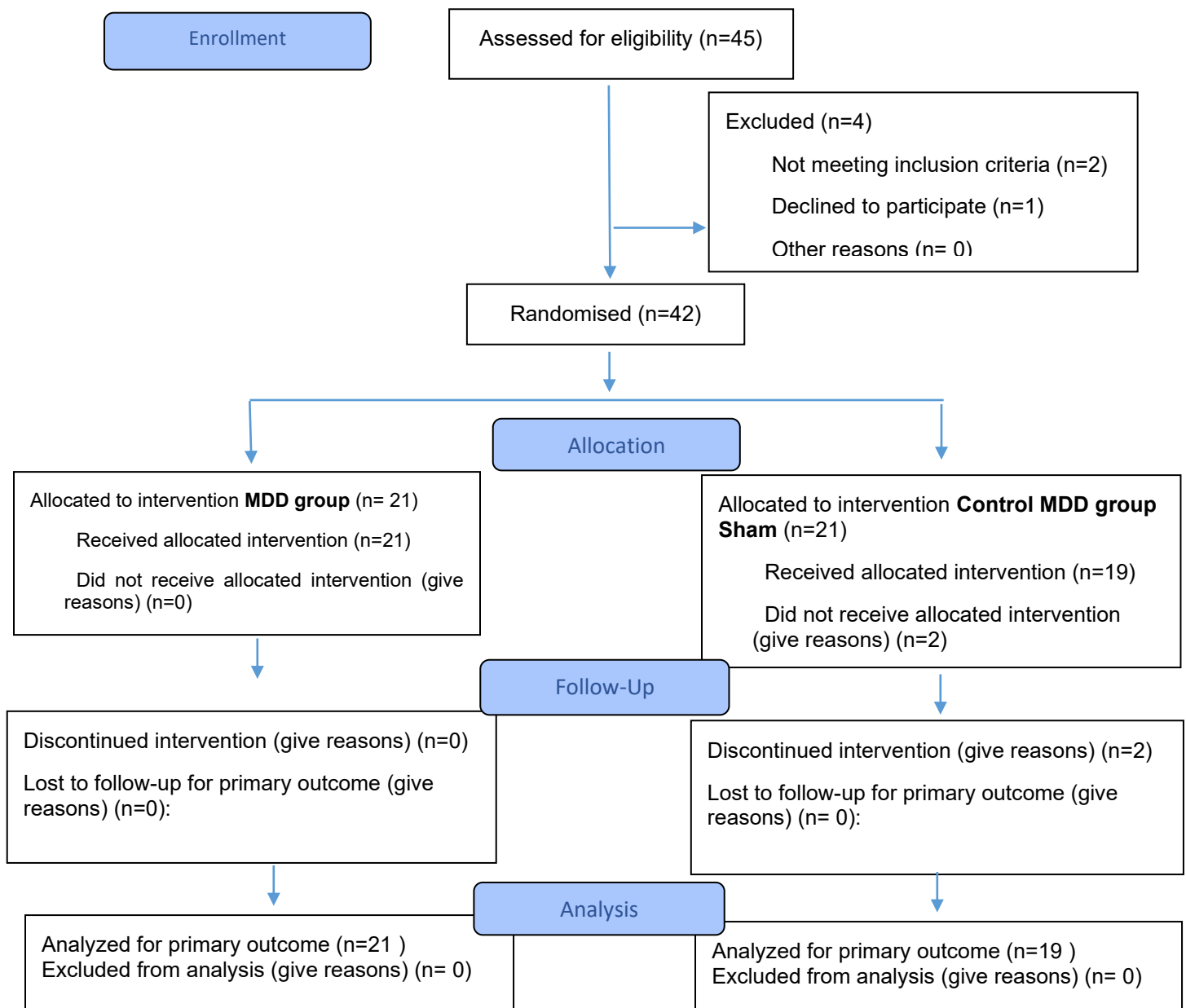
Materials and Methods

This was a randomized, parallel-group, block-randomized, single-blind clinical trial. The protocol was approved by the National Commission for Scientific Research under registration number (R-2024-785-075). Subjects were invited to participate in the psychiatry department of the Bajío No. 1 High-Specialty Medical Unit, IMSS. The participants signed an informed consent form explaining the process; participation was voluntary. The procedures performed on the participants were in accordance with international ethical standards for research involving human subjects, the Nuremberg Code, and the Declaration of Helsinki.

They were evaluated according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) by a qualified psychiatrist to confirm the diagnosis of major depressive disorder. Blinding was performed in patients who did not know whether or not they would receive treatment with low-intensity transcranial magnetic stimulation (Li-TMS).

The groups were selected at random, sequentially numbered. Each group consisted of 20 patients. The inclusion criteria were as follows: participants aged 18 to 60 years of both genders, a diagnosis of major depressive disorder (MDD) previously established by the treating psychiatrist, and the presence of insomnia as assessed by the Pittsburgh Sleep Quality Index (PSQI). The exclusion criteria included a history of epilepsy, schizophrenia, or neurosurgery; the presence of metal implants in the skull, neck, chest, or shoulder; pacemaker implantation; hormone replacement therapy; and pregnancy. The elimination criteria were patients who did not attend all their evaluations and did not complete 80% of all their intervention sessions, who presented with repetitive, intolerable symptoms, who withdrew from the study, and who did not continue with their pharmacological treatment. All participants were clinically evaluated by a qualified psychiatrist using the Hamilton Depression Rating Scale (HAM-D-17), the Pittsburgh Sleep Quality Index (PSQI), and the Patient Health Questionnaire-9 (PHQ-9) both prior to the first session and following the 20th Li-TMS session (Fig. 1). Adverse events were collected from all subjects at the end of all Li-TMS sessions.

Figure 1. Flow diagram of the progress through the phases of a randomised trial of two groups (that is, enrolment, intervention allocation, follow-up, and data analysis).



All participants consented to undergo complete blood count and biochemical testing at 7 a.m. on the first day following admission to the hospital unit. They provided 3 mL of venous blood in a container with EDTA anticoagulant and 4 mL in a container without anticoagulant for the determination of BDNF, CRP, cortisol, and metabolic markers,

including glucose, triglycerides, total cholesterol, and HDL. Prior to and following the end of the 20th Li-TMS session.

Following items of blood cytology were measured: neutrophil count, hemoglobin, lymphocyte count, leukocyte count, erythrocyte count, erythrocyte hematocrit, platelet count. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio were calculated. All blood samples were sent to laboratory of UMAE 1 Bajío and analyzed through LH 780 Hematology Analyzer of Beckman Coulter Inc

Determination of BDNF in serum

Serum BDNF levels were measured using the BDNF ELISA kit “MB2019079” (enzyme-linked immunosorbent assay), which is an in vitro immunoassay used for quantitative measurement of human BDNF in serum, plasma, cell culture supernatant, and urine. Manufactured by IBL International, it has high specificity due to its lack of reactivity with any other cytokine. For humans, 96-well sandwich type from the MyBioSource laboratory, with a detection range of 31.2-2000 pg/ml, sensitivity < 12.1 pg/ml, intra-assay precision <10% and inter-assay <12%.

Treatment with Li-TMS.

Participants received 20 sessions of Li-TMS with the medical device Nibbot Pro Series. Each Li-TMS session consisted in positioning an eight-shaped coil over the left dorsolateral prefrontal cortex (L-DLPFC) which dispensed magnetic pulses at 25 Hz, (30 mT) for 45 minutes. Subjects received one Li-TMS session daily, from Monday through Friday. The experimental group received conventional drug treatment combined with Li-TMS, and the control group were administered standard drug therapy

with coil placement, without exposure to Li-TMS emission. Both groups had the device coils placed on them. At the end of the study, the control group received Li-TMS treatment.

Statistical Analysis

A comparison will be made between the pre- and post-treatment groups. If the data are normally distributed, the paired t-test will be used to compare metabolites before and after the intervention; if the data are nonparametric, the Wilcoxon test will be used. Correlation analyses will be performed using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-normally distributed data. Additionally, linear and multiple regression analyses will be performed using the significant variables identified in the correlation analysis, along with the covariates of age, sex, education level, occupation, time since diagnosis, treatments used, and treatment adherence, using the stepwise method. The efficacy of the intervention will be evaluated based on the reduction in depressive symptoms assessed using the Hamilton Depression Rating Scale-17 and insomnia using the Pittsburgh Insomnia Questionnaire at the end of treatment with EMTbi. A p-value of <0.05 will be considered statistically significant. Results will be analyzed using the Statistical Package for the Social Sciences (SPSS version 21). Patient data records are stored at the Clinical Epidemiology Research Unit. OOAD Guanajuato. Mexican Institute of Social Security. León, Guanajuato, México. There were no missing data points in the statistical analysis.

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