Altered Amplitude of Low-Frequency Fluctuations of rs-fMRI Signal followed by rTMS Analgesic Effects in Non-Specific Chronic Low Back Pain (CLBP) Patients

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ABSTRACT

Background: Non-specific chronic low back pain (CLBP) is a common painful condition and is responsible for different physical disorders. Despite alternative therapies, patients still suffer from persistent pain. Repetitive transcranial magnetic stimulation (rTMS) has provided much evidence of pain reduction, but results have not been examined deeply in CLBP symptoms.

Objective: The analgesic effect of rTMS in non-specific CLBP patients was evaluated by the amplitude of low-frequency fluctuation (ALFF) analysis in resting-state fMRI.

Material and Methods: In this experimental study, fifteen non-specific CLBP participants (46.87±10.89 years) received 20 Hz rTMS over the motor cortex. The pain intensity and brain functional scan were obtained during pre and post-stimulation for all participants. The ALFF maps of the brain in two scan sessions were identified and the percentage of pain reduction (PPR%) was determined using paired t-test. Also, correlation analysis was used to find a relationship between ALFFs and pain intensity.

Results: Pain intensity was significantly reduced after induced-rTMS in non-specific CLBP (36.22%±13.28, $P<0.05$). Positive correlation was found between ALFF in the insula (INS) and pain intensity ($r_{pre\text{-}rTMS}=0.59$, $r_{post\text{-}rTMS}=0.58$) while ALFF in medial prefrontal cortex (mPFC) and pain intensity had negatively correlated ($r_{pre\text{-}rTMS}=-0.54$, $r_{post\text{-}rTMS}=-0.56$) ($P<0.05$). ALFF increased in mPFC while INS, thalamus (THA), and supplementary motor area (SMA) showed decremental ALFF followed by rTMS.

Conclusion: This study demonstrated that ALFF in INS, THA, mPFC, and SMA is associated with CLBP symptoms and analgesic effects of rTMS. ALFF potentially seems to be a proper objective neuroimaging parameter to link spontaneous brain activity with pain intensity in non-specific CLBP patients.

Keywords

Low Back Pain; Pain Relief; Disability Evaluation; Transcranial Magnetic Stimulation; Functional MRI

Introduction

Chronic low back pain (CLBP) with no clear clinical evidence which is known as non-specific is a highly prevalent pain condition and is a leading cause of temporary or permanent physical and even psychological disorders worldwide [1]. Despite alternative

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therapies such as pharmacological prescriptions, exercise programs, bed rest, acupuncture, spinal manipulation, or even surgery and injection, patients with CLBP still suffer from persistence or recurrence of pain and movement problems as serious difficulties in daily life [2-5]. In this context, high frequency repetitive transcranial magnetic stimulation (rTMS) as an innovative, safe, and noninvasive alternative technique has been recently proposed for possible use in chronic pain treatment [6]. In the TMS method, an electric current is rapidly changed and flows through an electromagnetic coil that has been used to produce short magnetic pulses that pass easily and painlessly through the brain. These pulses induce an electric field that results in neuron depolarization and cortical stimulation that has been applied to modulate altered brain activities to alleviate pain [7, 8].

Different studies showed that a single session of high-frequency rTMS over the primary motor cortex (M1) results in pain relief for several days (up to a week) after stimulation that the optimal effect is delayed by 2-4 days [9-12]. Nevertheless, the neural mechanisms that contribute to the pain reduction effects of rTMS for patients with pain are still not completely understood. However, the modulation of neuronal plasticity and cortical excitability after induced high-frequency rTMS has been recently observed in functional neuroimaging studies [13, 14]. In this regard, the development of imaging techniques such as resting-state functional magnetic resonance imaging (rs-fMRI) can be used as a noninvasive method to investigate neural mechanisms involved with rTMS in humans [11].

Recently, accumulating evidence suggests that low-frequency (0.01-0.08 Hz) spontaneous blood oxygen level-dependent (BOLD) fluctuations in resting-state fMRI (rs-fMRI) provide physiological insights into neural spontaneous activity [15, 16]. The amplitude of low-frequency fluctuations (ALFFs) as a trustworthy method identifies the voxel-level spontaneous activity in the brain region. ALFF can measure the amplitude values of low-frequency oscillations (LFOs) of rs-fMRI time courses in the cortical regions and focus on the neural processes and activity in different brain regions rather than time-series similarities among regions [17, 18]. Importantly, disrupted LFOs amplitude has been reported in various clinical disorders including attention-deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder (OCD), Alzheimer's disease, schizophrenia, depressive disorders, and different pain symptoms [15, 16, 19-25]. Specifically, abnormalities in spontaneous brain activity in CLBP patients have been revealed in widespread brain regions such as the medial prefrontal cortex (mPFC), supplementary motor area (SMA), anterior cingulate cortex (ACC), precuneus (PC), amygdala (AMY), thalamus (THA), and insula (INS) [18].

Although there is a study to evaluate the effect of the acupuncture technique on the low-frequency BOLD signal oscillation response in the insular in patients with CLBP [26], no study has not been existed to investigate ALFF changes followed by rTMS application in different brain regions of CLBP patients. Therefore, the present study for the first time aimed to determine the regional spontaneous BOLD signal fluctuations and pain alterations followed by rTMS in non-specific CLBP patients.

Material and Methods

Participants

Fifteen (7 males, 8 females) right-handed non-specific CLBP patients, originally recruited from Tehran University of Medical Sciences (TUMS) hospitals, participated in this experimental study. The inclusion criteria were that all patients would be aged between 20 and 60 years with a disease duration of more than three months, and also experienced pain intensity of at least 4 (Visual Analog Scale/Score (VAS)=0-10) in the week before the first rTMS session. Patients were excluded if they

had potential contraindications for the use of TMS or MRI such as a history of seizures, claustrophobia, brain trauma, brain surgery, a pacemaker, or other metallic implants.

Ten right-handed healthy control (HC) voluntaries (4 males, 6 females) also participated in this study. The inclusion criteria were that all subjects would be aged between 20 and 60 years without any history of chronic pain, and also be able to undergo MRI. Written informed consent was obtained from all participants including patients and HCs.

Study Paradigm

As shown in Figure 1, this was a pre-post study design. Resting-state fMRI was designed to evaluate the effect of brain stimulation of rTMS as a state-of-the-art pain relief method on spontaneous neural activity in non-specific CLBP patients. Two fMRI scanning sessions, before and after induce of the brain stimulation, were obtained. This study focused on the low frequency of BOLD signals. At each study session (i.e., before and after the brain stimulation), subjects were given questionnaires by Roland–Morris (R-M) to assess pain-related disability, also the conventional method of VAS to determine the pain intensity.

Brain Stimulation

All participants underwent a brain rTMS stimulation in the first session of the study. The rTMS was performed via an eight-shaped coil (MC-B65-HO butterfly shape coil MagVenture) connected to a magnetic stimulator (MagPro X100, MagVenture) placed over the motor cortex corresponding to the M1 brain region. The stimulation parameters provided a frequency of 20 Hz with a field intensity of 95% of motor threshold (MT) and trains of 40 pulses (in 2 sec), followed by a 28 sec rest period. It should be noted that MT was defined as the lowest intensity that yielded motor evoked potentials (MEPs) in 50% of consecutive trials for each participant obtained with the coil located on the hand area of the left hemisphere under TMS conditions until observed the muscle contraction of the right hand.

Pain Evaluation

To measure the pain intensity experienced by the CLBP patients, a VAS was used. The VAS comprised a horizontal line of 10-cm which no pain (0) indicates on the left side (start point of the line) and the strongest pain imaginable (10) at the right side (the end point of the line). A ruler was used to measure the pain intensity

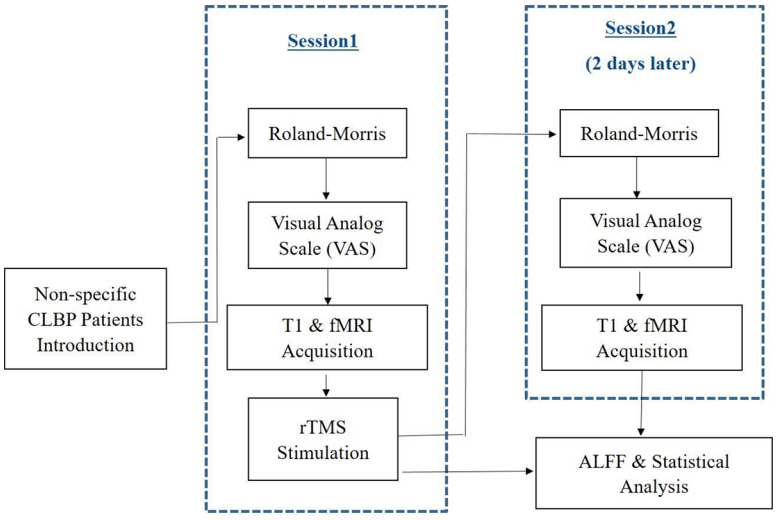

Figure 1: Experimental protocol: The experiment consisted of two sessions of fMRI before and 2 days after rTMS for every subject. Pain intensity and disability score were assessed every session. CLBP: Chronic low back pain, fMRI: Functional magnetic resonance imaging, rTMS: Repetition transcranial magnetic stimulation, ALFF: Amplitude of low-frequency fluctuation

by the measured length (in centimeters) from the start point to the point marking a cross on the VAS by each patient. The pain intensity was again determined within 2 days of completing the rTMS session using the same procedures described above. The percentage of pain reduction (PPR) was obtained from the measurement of VAS scores at pre and post rTMS sessions according to the following equation [10]:

$$PPR = \frac{VAS_{pre} - VAS_{post}}{VAS_{pre}} \times 100 \quad (1)$$

Disability Evaluation

To measure the disability scale, a self-administered questionnaire of Roland-Morris (RM) with a 24-point scale was used in which greater levels of disability were linked directly with the higher numbers on it. The RM is sensitive to change over time for low back pain patients. Participants were asked to mark next to every appropriate statement on a 24-point scale. To get a disability score for non-specific CLBP patients, add up all the marked statements. The disability was again determined within 2 days of completing the rTMS session using the same procedures described above. The percentage of disability change (PDC) was calculated from the Roland–Morris (RM) questionnaire scores measured at pre and post rTMS sessions according to the following equation:

$$PDC = \frac{RM_{pre} - RM_{post}}{RM_{pre}} \times 100 \quad (2)$$

Imaging Data Acquisition

MRI data were acquired using a 3.0 Tesla Siemens, MAGNETRON Prisma (Munich, Germany) scanner with a standard 64-channel radio-frequency head coil. For each subject, a high-resolution T1-weighted structural image for registration purposes was collected using a magnetization-prepared rapid gradient-echo imaging (MPRAGE) with repetition time/echo time=2300/2.32 ms; FOV=240 mm×240 mm; matrix size=256×256; voxel size =0.9×0.9×0.9 mm^3 and flip angle=8°. During the fMRI scan, all participants were asked to lie in a supine position in the scanner with their eyes closed, without falling asleep, and not to think about anything in particular. Their heads were fixed to minimize head motion using pillows and foam pads. T2*-weighted resting fMRI scans were acquired using a gradient recalled echo echo-planar imaging (GRE-EPI) sequence with repetition time=3000 ms; echo time=40 ms; flip angle=90°; number of slices=42; field of view=220×220 mm^2 without gap, matrix size=64×64; voxel size=3.4×3.4×3 mm^3, slice thickness=3 mm; and 200 volumes.

fMRI Signal Analysis

Resting-state fMRI data were preprocessed using the Data Processing & Analysis for Brain Imaging (DPABI v6.0, http://www.rfmri.org/dpabi) toolbox for SPM12 (Wellcome Department of Cognitive Neurology. London, UK) running in MATLAB 2017b (Mathworks, Inc., Natick, MA, USA) with these steps: for stabilization, the first 10 time points were discarded. Then slice timing correction, motion correction, registration, and normalization in Montreal Neurological Institute [MNI] 152 space, and spatial smoothing with 6 mm FWHM were respectively performed. It should be mentioned that the exclusion criteria for the max head motion in the motion correction step were 3 mm for translation and 3° for rotation motion. Also, in the normalization step, T1-weighted high-resolution images were registered to the mean fMRI images, and the resulting images were segmented and transformed into standard MNI spaces using the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra toolbox (DARTEL) [17].

ALFF Computing

For ALFF analysis, a preprocessed BOLD time series $x(i)=x(t_i)$, i=1, 2, …, N with N time points can be written in the frequency domain

as:

$$x_i = \frac{1}{N}\sum_{k=1}^{N} X_k e^{\frac{2\pi j}{N}ki}, i = 1,2,\ldots,N \quad (3)$$

Where the spectral amplitude terms, $X_k=x(f_k)$ with k=1, 2, …, N are the discrete Fourier transformed coefficients. Then, the power spectrum (PS) can be calculated at the frequency band of 0.01<f<0.08 Hz to reduce the low-frequency drift effects and high-frequency physiological noise in each voxel.

$$PS(0.01,0.08) = \frac{1}{N}\sum_{k=0.01}^{0.08} |X_k|^2 \quad (4)$$

Since each term in Eq. 4 is proportional to the square of the amplitude at the frequency *k*, ALFF can be computed using the following equation (i.e., the square root of the PS density at each frequency component) [17, 21, 22]:

$$ALFF = \frac{1}{\sqrt{N}}\sum_{k=0.01}^{0.08} |X_k| \quad (5)$$

Statistical Analyses

Individual-level values were analyzed using the Statistical Package for Social Science (SPSS, v. 22.0, IBM Corporation, Armonk, NY). The characteristics variables were performed using descriptive analysis (mean±SD) for quantitative variables. Percentage of pain reduction and percentage of disability changes were compared within the/a patient group using paired t-test analysis, with the statistical significance levels set at $P \leq 0.05$.

DPABI V6.0 was used for statistical analysis on ALFF. Different types of t-test statistical analysis were used to compare the values in groups. Multiple comparison corrections were performed based on Gaussian random field (GRF) theory (voxel wise $P<0.01$, cluster-wise $P<0.05$, and two-tailed test).

Finally, the correlation analysis was used to explore the relationship between ALFF values in brain areas and VAS scores in pre and post-stimulation sessions.

Results

Table 1, shows the descriptive statistics for general demographics data and pain information of CLBP participants and healthy control.

The measured pain intensity and disability score for non-specific CLBP patients in every session are presented in Table 2. The mean pain intensity changed significantly from

Table 1: Demographics for non-specific CLBP (Chronic low back pain) patients and HCs (Health control). The values presented are 'mean (standard deviation)' for characteristics.

Characteristics	Age (year)	Female/Men (%)	Pain Duration (year)	*BMI (Kg/m²)
Non-specific CLBP (N=15)	44.87 (10.89)	54/46	1.88 (1.10)	27.39 (4.59)
HC (N=10)	40.88 (8.73)	60/40	---	23.70 (5.46)

N: Number of participants, CLBP: Chronic low back pain, HC: Health control, BMI: Body mass index, *Normal BMI: 18.5-25, more than 25 is overweight, less than 18.5 is underweight.

Table 2: Measured pain and disability results before and after rTMS (Repetition transcranial magnetic stimulation) stimulation in CLBP (Chronic low back pain) patients.

Non-specific CLBP (N=15)	VAS Score (#) Mean±SD	R-M Score (#) Mean±SD
Pre Stimulation	7.25±0.99	15.58±3.40
Post Stimulation	4.60±1.03	13.25±3.46
PPR% or PDC%	36.22±13.28	15.89±7.82

CLBP: Chronic low back pain, VAS: Visual analysis scale, R-M: Roland-Morris, SD: Standard deviation, PPR: Percentage of pain reduction, PDC: Percentage of disability change

7.25±0.99 in the first session to 4.60±1.03 in the second session (i.e, 2 days after brain stimulation using rTMS) in the patient group (t=6.80, P<0.001). The mean percentage of pain reduction (PPR) was 36.23%±13.28. The mean disability score was 15.58±3.40 before brain stimulation while it was 13.25±3.46 after stimulation (t=12.72, P<0.001). The mean percentage of disability change (PDC) was 15.89%±7.82.

Figure 2 shows the ALFF analysis process from non-specific CLBP patients in rs-fMRI data of the pre and post-rTMS stimulation sessions. Figure 2 (A) demonstrates the BOLD signal was extracted from a 6 mm diameter ROI (e.g., INS (L)) centered on the peak coordinates (-47, 11, -5) during the scan time. The time series were transformed into the frequency domain with a Fast Fourier Transform (FFT) (Figure 2 (B)) and the power spectrum was obtained (Figure 2 (C)). A filtered power spectrum in the frequency band of 0.01 Hz to 0.08 Hz was used to measure ALFF (Figure 2 (D)). The power of a frequency spectrum is proportional to the square of the amplitude of this frequency component, so, the square root was calculated at the frequency band (0.01–0.08 Hz) to measure the average of the square root as ALFF in this region (Figure 2 (E)).

Accordingly, Figure 3 and Table 3 show the ALFF analysis results in the HC group and non-specific CLBP patients before and after brain stimulation of rTMS.

Subsequently, paired t-test analysis was performed to find ALFF differences before and after rTMS in non-specific CLBP patients, and a two-sample t-test was used to find differences between patients and HCs (Figure 4).

The correlation analysis was used to explore the logical potential relationship between the pain intensity in pre/post-stimulation sessions and the ALFF values in brain regions. As shown in Figure 5, the Pearson correlation coefficient between VAS and ALFF in mPFC in the pre-rTMS session and the post-rTMS session was -0.54 and -0.56, respectively. In addition, the Pearson correlation coefficient between VAS and ALFF in the INS (L) in the pre-rTMS session is 0.59 whereas this is 0.58

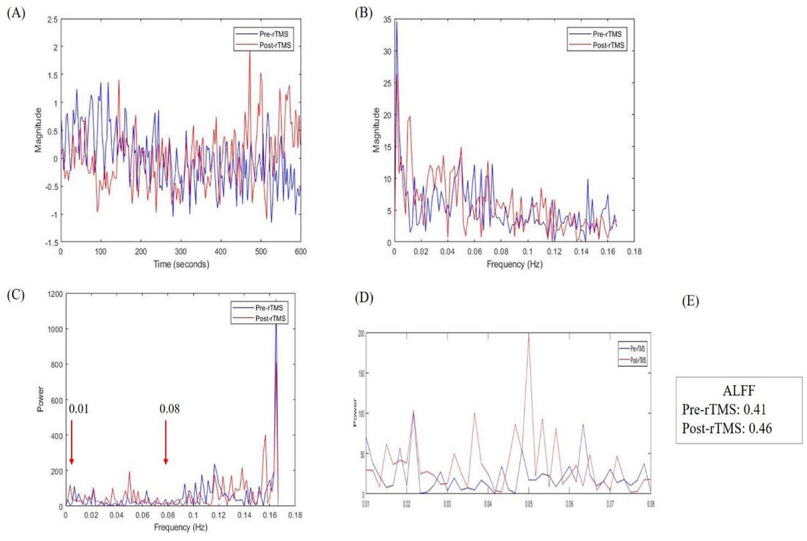

Figure 2: ALFF analysis for pre (blue) and post (red) rTMS sessions: (A) Time series of an individual ROI (INS (L)), (B) transformed signal by FFT, (C) power spectrum of the signal, (D) filtered power spectrum in 0.01 to 0.08, (E) power spectrum between 0.01 and 0.08 Hz, i.e., ALFF. rTMS: Repetition transcranial magnetic stimulation, ROI: region of interest, INS: Insula, FFT: Fast Fourier Transform, ALFF: Amplitude of low-frequency fluctuation

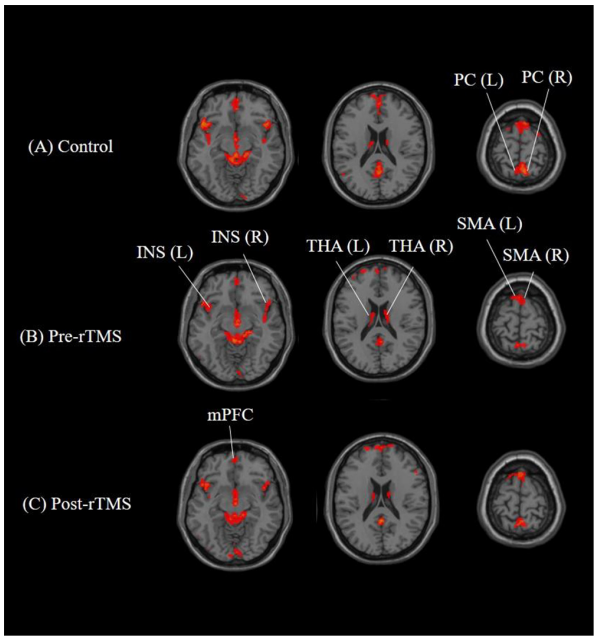

Figure 3: ALFF maps: Axial view of the results from the ALFF analysis (averaged across subjects) in (A) healthy control group (B) patient group at the first session before rTMS and (C) patient group at second session in 2-4 days after rTMS session (P<0.05). Warm colors indicate increased ALFF in different brain regions. ALFF: Amplitude of low-frequency fluctuation, rTMS: Repetition transcranial magnetic stimulation, INS: Insula, mPFC: Medial prefrontal cortex, THA: Thalamus, SMA: Supplementary motor area, PC: Precuneus

in the post-rTMS session (Figure 5).

Discussion

The major aim of this study was to investigate the differences in the regional spontaneous activity of the brain in non-specific CLBP patients before and after rTMS brain stimulation by using ALFF analysis. Although disrupted low-frequency oscillation amplitude has been reported in various clinical disorders relative to healthy control [19-22, 25], neural modulation effects investigation using ALFF analysis is a new approach in neuroimaging chronic pain symptoms studies. Therefore, in the present study, the ALFF analysis was applied to evaluate the alterations of spontaneous neural activity in non-specific CLBP patients after induced rTMS.

The results of rs-fMRI imaging data analysis in the present study after induced-rTMS compared with pre-stimulation in non-specific CLBP patients, initially demonstrated changes in spontaneous brain activity of the bilateral INS, bilateral THA, bilateral SMA, bilateral PC, and mPFC. Although the mechanism of brain changes after rTMS has not been clearly known yet, some previous imaging studies demonstrated that M1 stimulation by

Table 3: ALFF (Amplitude of low-frequency fluctuation) analysis results derived in HC (Health control) group and non-specific CLBP (Chronic low back pain) patients.

Region	Peak MNI			T-value	T-value	T-value
	X	Y	Z	HC	Pre-stimulation	Post-stimulation
INS (L)	-47	11	-5	3.89	5.44	3.80
INS (R)	46	15	-5	3.81	5.02	3.83
SMA (L)	0	23	65	3.91	5.06	3.71
SMA (R)	3	23	65	3.98	4.38	3.64
THA (L)	-12	-14	20	4.63	4.52	3.79
THA (R)	9	-6	15	5.41	6.37	3.90
PC (L)	-6	-55	70	4.33	4.02	2.81
PC (R)	5	-60	70	5.55	3.05	3.73
mPFC	1	55	-3	4.81	4.69	5.75

MNI: Montreal Neurological Institute, HC: Health control, INS: Insula, SMA: Supplementary motor area, THA: Thalamus, PC: Precuneus, Mpfc: Medial prefrontal cortex

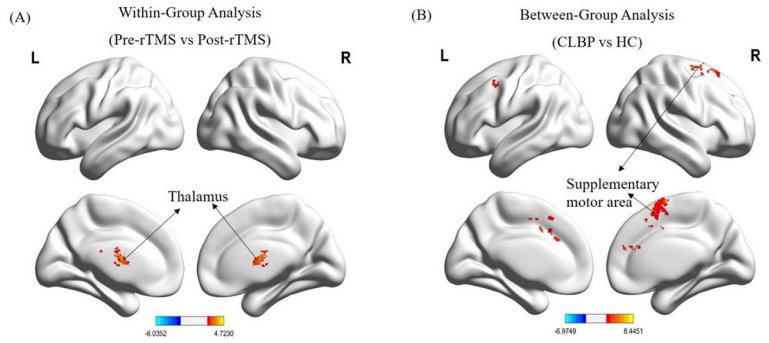

Figure 4: ALFF group analysis. (A) Within-group analyses of the ALFF in non-specific CLBP patients before and after rTMS. (B) Between-group analyses of the ALFF between patients and HC groups (two-tailed, voxel-level *P*<0.01; GRF correction, cluster-level *P*<0.05). CLBP: Chronic low back pain, HC: Health control, ALFF: Amplitude of low-frequency fluctuation, rTMS: Repetition transcranial magnetic stimulation, GRF: Gaussian random field

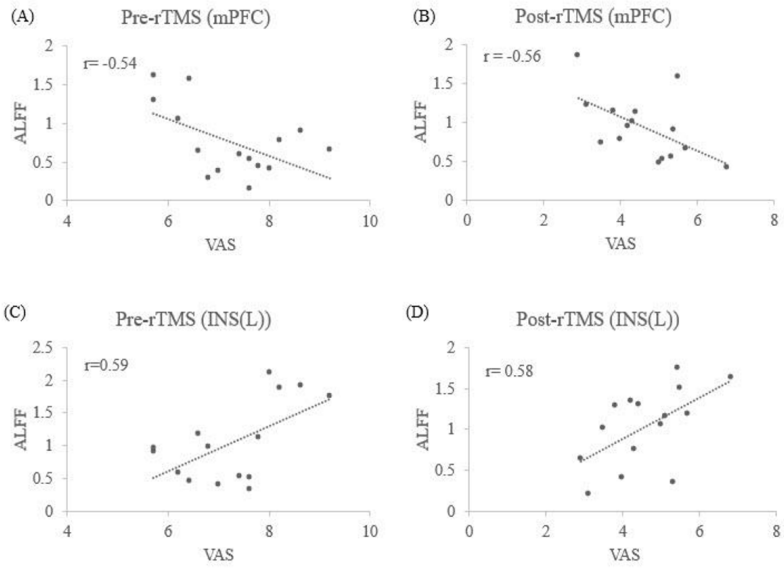

Figure 5: Correlation analysis of ALFF and pain intensity. (A) The negative correlation between pain intensity (i.e., VAS) and ALFF in mPFC before rTMS stimulation (Pearson correlation, r=-0.54), (B) the negative correlation between VAS and ALFF in mPFC after rTMS stimulation (Pearson correlation, r=-0.56), (C) the positive correlation between pain intensity (i.e., VAS) and ALFF in INS (L) before rTMS stimulation (Pearson correlation, r=0.59), (D) the negative correlation between VAS and ALFF in INS (L) after rTMS stimulation (Pearson correlation, r=0.58). ALFF: Amplitude of low-frequency fluctuation, mPFC: Medial prefrontal cortex, INS (L): Left insula, rTMS: Repetition transcranial magnetic stimulation, VAS: Visual analysis scale.

rTMS resulted in the brain activity alteration not only in the motor system but also in the regions which are related to pain processing and modulation [13, 27]. Importantly, in the present study, decreased ALFF was observed in bilateral INS, bilateral THA, and bilateral SMA after using high-frequency rTMS for patients suffering from CLBP. In contrast, ALFF

of mPFC increased after brain stimulation in the patient group (Table 3). Besides, THA showed a notable difference in pre and post-rTMS brain stimulation sessions in CLBP patients (Figure 4). Specifically, INS, THA, and prefrontal cortices have been introduced as essential brain areas in the pain matrix that disrupted activation in these regions is associated with the experience of pain [28]. Therefore, in line with recent studies, these brain regions that are sensitive to pain intensity changes may have the potential for measurement of treatment response in CLBP patients [18]. In this regard, consistent with our findings, decreased spontaneous neural activity in INS simultaneous with decreased pain intensity in CLBP patients after ankle acupuncture was observed [26]. Despite applying different pain reduction methods in that study (using acupuncture) with our study (using rTMS), it seems that INS is a major brain region in response to the sense of pain in patients suffering from CLBP. However, no study exists to investigate spontaneous neural activity changes followed by rTMS in non-specific CLBP patients.

Another major concern in our study is that the spontaneous activation of brain regions demonstrated differences between CLBP patients and healthy control. Actually, CLBP patients showed the higher amplitude of low-frequency oscillation values in bilateral SMA and bilateral INS relative to HC while the lower ALFF was observed in bilateral PC and mPFC (Table 3). Some previous studies also found the augmented activation in the SMA in chronic low back pain patients compared with HC, thereby, SMA has been suggested to discriminate CLBP subjects from HCs [18]. Similarly, in our study, SMA showed a significant difference in non-specific CLBP compared with HC (Figure 4). Moreover, the mPFC and PC are important regions in the default mode network (DMN) and the INS is an essential region in the salience network (SN). DMN is disrupted in CLBP and involved in the spontaneous disengagement of pain attention and is suppressed in pain presence [18, 29]. In addition, decreased ALFF was observed in the PC in CLBP patients compared with HC which is under another low back pain study. However, they induced acute LBP by injection of hypertonic saline into the back muscles of the healthy subjects [24]. Despite the difference in the kind of LBP (being acute or chronic), the decreased spontaneous BOLD signal in PC was observed in both conditions. On the other side, the salience network (SN) as one of the important brain responsive regions in CLBP which monitors sensory input alterations, is abnormal in CLBP [26, 29].

On the clinical side, the result of the current study in agreement with plenty of research has shown to decrease significantly in VAS pain scores after a single session of high-frequency rTMS in chronic pain patients [9, 10, 12, 30]. Indeed, the mean analgesic effect, measured with the VAS, in CLBP participants was 36.22% after high-frequency rTMS of M1 stimulation. This result is completely consistent with other reports that are expected to decrease pain intensity by 20% to 45% after active brain stimulation using high-frequency rTMS of M1 [7, 12, 13, 31, 32]. So, this result also supports the idea of the beneficial role of rTMS in pain management.

Furthermore, the results of the present study demonstrated a significant correlation between ALFF of mPFC and pain intensity in CLBP patients ($P<0.05$) (Figure 5). Pearson correlation analysis revealed an indirect correlation between mPFC ALFF values and VAS scores in CLBP patients in the pre and post-stimulation of -0.54 and -0.56, respectively. Recent studies also found the ALFF values in default mode network (DMN) regions are negatively correlated to the pain intensity in CLBP patients and also in patients with discogenic low back and leg pain [17, 18]. Therefore, under these studies, the correlation analysis showed a possible relationship between the spontaneous brain activity of mPFC as the main part of DMN and the pain intensity of CLBP in

two sessions of imaging before and after induced-rTMS. This result highlighted the role of frontal regions of the brain in the cognitive dimension of pain that is reported in some previous research [33]. Moreover, a direct correlation between the ALFF in the INS (L) and pain intensity in pre and post-stimulation sessions was obtained in the present study ($r_{pre\text{-}rTMS}$=0.59, $r_{post\text{-}rTMS}$=0.58) (Figure 5). Inconsistent with our finding, a positive correlation between the VAS score of CLBP patients and ALFF values in INS (L) was explored in some previous studies [18, 26]. The results support the idea that INS is an important part of the pain matrix and also have a multidimensional role in different aspect of pain including sensory, cognitive, and emotional [24, 26, 33-35].

Conclusion

In conclusion, altered spontaneous neural activity in different brain regions including the insula, thalamus, medial prefrontal cortex, precuneus, and the supplementary motor area may provide evidence to introduce ALFF as a non-invasive neuro biomarker to monitor CLBP symptoms. The results of correlation analyses potentially open up a new approach to link ALFF as a proper objective neuroimaging parameter with the VAS score as a classic subjective pain intensity evaluation.

Acknowledgment

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Authors' Contribution

M. Masoumbeigi performed the data acquisition, fMRI analysis, interpretation of data, and drafting of the work as the principal author. N. Riyahi Alam designed the main conception of this work and approved the final version to be published. R. Kordi and M. Rostami contributed to the recruitment and clinical assessment of the patients. A. Rahimi foroushani analyzed the results statistically. AH. Jafari and H. Hashemi revised the manuscript critically for important intellectual content. A. Ebrahimpour contributed to the data acquisition. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

The protocol of the human study was approved by the local ethical committee, Tehran University of Medical Sciences (TUMS), Tehran, Iran (Approval number: IR.TUMS.MEDICINE.REC.1397.957).

Informed Consent

Written informed consent was obtained from all participants including patients and HCs.

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Conflict of Interest

None

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