Amplitude changes in low frequency fluctuation in brains of primary dysmenorrheal treated by acupuncture: a resting-state fMRI study

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Abstract: Objective: The aim of this study was to observe changes in amplitude of low frequency fluctuation (ALFF), before and after acupuncture of the points of Sanyinjiao and Diji, with resting-state fMRI (rsfMRI) technology, investigating central analgesic mechanisms of acupuncture of the points of Sanyinjiao and Diji in patients with primary dysmenorrhea. Methods: A total of 16 primary dysmenorrhea-eligible patients underwent rsfMRI scanning with a feeling of pain on the first or second day of onset of the menstruation period. Anatomical images scanning, resting-state scanning (R1), stimulating-acupoints scanning (AP), and resting-state scanning (R2) after withdrawing the needles were used. Data were analyzed using ALFF. Moreover, pain scores, before, during, and after acupuncture, were evaluated according to VAS. Results: (1) After acupuncture treatment, the pain level decreased from 10 to 8. It further decreased to 4 after treatment; (2) Two-sample paired t-tests indicated brain regions with differences in ALFF between AP and R1. Increased ALFF (AP > R1) appeared in the bilateral medial frontal gyrus, bilateral superior frontal gyrus, bilateral anterior cingulate gyrus, and collosum; (3) Two-sample paired t-tests indicated brain regions with differences in ALFF between R2 and AP. Decreased ALFF (R2 < AP) appeared in the bilateral cingulate gyrus, bilateral anterior cingulate gyrus, insula, hippocampus, thalamus, lentiform nucleus, midbrain, pons, and cerebellum. Conclusion: Acupuncture points of Sanyinjiao and Diji play an important role in relieving pain and increasing or decreasing of ALFF in a series of pain-related encephalic regions. These encephalic regions may be a target of the analgesic effects of acupuncture. Moreover, these may be the conceivable central analgesic mechanisms of acupuncture of the points of Sanyinjiao and Diji for patients with primary dysmenorrhea.

Keywords: Primary dysmenorrhea, acupuncture, resting-state functional magnetic resonance imaging, amplitude of low frequency fluctuation (ALFF)

Introduction

Dysmenorrhea is a common and frequently-occurring disease in gynecology. Primary dysmenorrhea (PD) or functional dysmenorrhea are parts of this disease without reproductive organ lesions [1]. Primary dysmenorrhea is one of the most common menstrual disorders among female college students, affecting daily lives and study habits. In general, incidence of PD among college girls is on the rise [2]. PD has attracted much attention due to its high incidence.
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tion (ALFF), before and after acupuncture of the points of Sanyinjiao and Diji, in patients with primary dysmenorrheal adopting resting-state fMRI technology, investigating the central analgesic mechanisms of acupuncture of the points of Sanyinjiao and Diji. All data were analyzed using ALFF.

Materials and methods

Subjects

There were 16 PD patients, studying at Henan University of TCM, included in the current study. All patients were in accordance with the following inclusion criteria: (1) In accord with diagnostic criteria (refers to the 1993 edition the Guiding Principles of Clinical Research in the Treatment of Chinese New Medicine in Dysmenorrhea by the Bureau of Drug Administration of the Ministry of Public Health of PRC and Gynecology in college teaching material); (2) Between 19 and 30 years old without bearing a baby; (3) Regular menstruation (28 ± 7 d); (4) No acupuncture treatment in the previous 3 months and no paregoric, sedatives, or hormones within 2 weeks of this trial; (5) Total points of CMSS ≥10; (6) VAS score ≥4; (7) Dextro-manual; and (8) Provided informed consent. Exclusion criteria: (1) Patients combined with severe life-threatening diseases and mental illness; (2) Pregnant or preparing for pregnancy; and (3) Patients contraindicated with acupuncture or MRI scanning.

Methods

Acupuncture method: Researchers located the bilateral sanyinjiao in the spleen meridian of foot-taiyin (SP6) and acupunctured the points when patients felt pain (sensations of tingling and throbbing). The professional acupuncturist used a 0.30 mm*40.00 mm needle (special and nonmagnetic; Great Wall of China Medical Supplies Factory (Jiangsu), batch number: 20010168). The needle was inserted vertically at sanyinjiao (SP6) about 1-1.5 cun, with the same operation to diji (SP8). After getting a Qi sensation, the needle was manipulated at each acupoint for about 0.5 minutes, with the method of twisting 60 times per minute and a range of about 180-360 degrees. Needles were then withdrawn without lifting, thrusting, or retaining. In the whole trail, all manipulations were done by one experienced acupuncturist. Pain scores were assessed according to visual analogue scores (VAS), using a 10-cm line with the description of “no pain” at one extreme (representing zero) and “intolerable pain” at the other extreme (representing ten). Participants were asked to rate the degree of pain by making a mark on the line. Pain scores were calculated by measuring the distance from zero to that mark using a ruler with a minimum measurement of one mm [6].

Scanning methods and data acquisition of rsfMRI: Structural and functional data of all subjects were collected by PHILIPS Achieva 1.5T MRI scanner of the First affiliated Hospital of Henan University of TCM. When the subjects achieved menstruation and felt pain, they followed these instructions: 1) Stick 1 grain of oleum morrhuae on the tip of the left ear to get the location to process data; 2) Plug the ear with a rubber plug to reduce the distraction of noise; 3) Lie flat on the scanning table, fixing the head after the subject feels at ease; and 4) Turn off the lights and tell the subject to be relaxed and clear-minded, with eyes closed and still. The trial was conducted under the conditions of audio-visual closed without lights. After scanning, subjects were asked about the acupuncture sensation and if there was head-movement, body-movement, or thinking activities, getting the picture of the scanning process. Each of the 16 subjects had obvious needle sensations, with no obvious head or body movements. Thus, all data was adopted for subsequent analysis.

Spin-echo sequence was adopted as the scanning parameter of anatomical imaging, while fast-card gradient echo train was adopted for functional imaging. For T1W anatomical images, the SE sequence was chosen with an axial view, flip angle 90-degree, TR 7.1 s, TE 3.2 s, and Matris 256*256. For functional images, EPI-BOLD sequence was used, with an axial view, flip angle 90-degree, TR 2000 ms, TE 50 ms, Matris 64*64, depth 5.0 mm, and gap 1 mm. Procedures included: Rest 1 (R1), acupuncture at bilateral sanyinjiao (SP6) and diji (SP8), Acupuncture (AP), and structural scan after withdrawing the needles (T) and Rest (R2).

Data processing and statistical analysis

Differences in pain scores were evaluated using Student’s t-test with two-tailed 95% confi-
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Results

Changes in pain scores before, during, and after acupuncture

After each acupuncture treatment, patients felt relaxed and reported less stiffness in general, as well as noticeable pain improvement in primary dysmenorrheal. As shown in Figure 1, compared with before treatment, pain scores during acupuncture treatment significantly decreased, further decreasing after treatment.

Variation conditions of brain regions according to comparison of AP with R1

Two-sample paired t-tests indicated brain regions with differences in ALFF between AP and R1. Increased ALFF (AP > R1) appeared in the bilateral medial frontal gyrus, bilateral superior frontal gyrus, bilateral anterior cingulate gyrus, and collosus (see Figure 2, Table 1).

Variation conditions of brain regions according to comparison of R2 with AP

Results of R2 and AP paired t-tests indicated that decreased ALFF (R2 < AP) appeared in the bilateral cingulate cortex, bilateral anterior cingulate cortex, insula, hippocampus, thalamus, lenticular nucleus, midbrain, pons, and cerebellum (see Figure 3, Table 2).

Discussion

Dysmenorrhea is a common and frequently-occurring diseases in gynecology, seriously impacting the daily lives of women. The clinical curative of acupuncture and moxibustion for primary dysmenorrhea is remarkable. At present, research on acupuncture mechanisms using rsfMRI has become a hot topic at home and abroad, with some achievements made. However, application of this technique in acupuncture treatment of primary dysmenorrhea is still rare.

The current study aimed to observe changes in amplitude of low frequency fluctuation (ALFF), before and after acupuncture of the points of Sanyinjiao and Diji in patients with primary dysmenorrheal, adopting resting-state fMRI tech-
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The ALFF algorithm obtains the power spectrum of the time series of each voxel signal in the brain from data processed by Fourier transform, calculating the ALFF of each voxel. ALFF has been considered to reflect spontaneous neural synchronization directly [7, 8], while spontaneous neural synchronization can reflect the interaction and neural network connection of each related brain region, to some extent. Therefore, the fMRI based ALFF algorithm can reflect a variety of neural activity and network connections of related brain regions in a resting state. This algorithm has been verified in studies concerning attention deficit hyperactivity disorder (ADHD) and differences of visual stimulation between eyes opening and closing [9-13], proving to be a credible algorithm [14].

Acupuncturing at points could make some brain regions activated or inhibited, reflecting the immediate effects of acupuncture. It also has after effects [15].

In the current study, results of the paired t-test of AP and R1 showed that brain regions with increased ALFF (reflecting the brain functional regions of immediate effect of acupuncture) were the bilateral medial frontal gyrus, bilateral superior frontal gyrus, bilateral anterior cingulate gyrus, and collosum. After withdrawing the needles, results of the paired t-test of R2 and AP showed that brain regions with decreased ALFF (reflecting the brain functional regions of after effect of acupuncture) were the bilateral anterior cingulate cortex, insula, hippocampus, thalamus, lenticular nucleus, midbrain, pons, and cerebellum. Xu Jianyang [16] adopted modified temporal clustering analysis to do the fMRI study concerning the after effects of acupuncturing at Hegu (LI4). It was found that, after 14 minutes, brain activity was still in a state of activating or inhibiting, which nearly matched the effects in the science of acupuncture and moxibustion. These activated brain regions also fit the phenomenon of after effects of acupuncture.

Acupuncture analgesia is related to the function of every part of the nerve system. Spinal marrow is the first step, dealing with and translating the information of acupuncture analgesia. The brainstem is the relay station, collating, analyzing, activating, synthesizing, and connecting the information. This has a significant effect in acupuncture analgesia [17]. In this study, considering the activating of midbrain and pons, it was speculated that acupuncture activates the thalamus, which is the element exchange station of pain and reaction due to the nociceptive information comes into cerebral cortex. Therefore, the para-fascicular nucleus and centro-median nucleus of intralaminar thalamic nuclei are very important centers of feeling and regulating pain. Results indicate that activating the centro-median nucleus could inhibit para-fascicular nucleus firing. When the centro-median nucleus is activated, it inhibits para-fascicular nucleus firing through 3 ways, cutting off the delivery of nociceptive information to the cerebral cortex [17]. The current study discovered that the bilateral thala-

Figure 2. Comparing AP and R1 in brain regions with obvious differences in ALFF (note: the left brain in this figure corresponds to subject’s right side, while the left in this figure corresponds to the right side; the clumps in this figure are not less than 10 voxel, voxel t > 2.262, uncorrected p < 0.05).
Table 1. Comparing AP and R1 in brain regions with significant differences in ALFF, peak coordinates, and cluster size

<table>
<thead>
<tr>
<th>Location</th>
<th>BA</th>
<th>Peak coordinates (mm)</th>
<th>Peak Z-value</th>
<th>Cluster size (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. medial frontal gyrus</td>
<td>6, 24, 32</td>
<td>0 -24 62</td>
<td>3.93</td>
<td>191</td>
</tr>
</tbody>
</table>

Figure 3. Comparing AP and R2 in brain regions with obvious differences in ALFF (note: the left brain in this figure corresponds to subject’s right side, while the left in this figure corresponds to the right side; the clumps in this figure are not less than 10 voxel, voxel t > 2.262, uncorrected p < 0.05).

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The cerebral cortex for information processing, eventually transforming into consciousness. The cerebral cortex is the highest center, which not only feels the information of pain but also participates in the modulation of pain. The cerebral cortex is not only a process of excitement and inhibition of acupuncture analgesia, but also a complex adjustment and command center. It can not only strengthen analgesia but also inhibit it. It plays the role of maintaining dynamic balance. Previous studies have found many connections between the prefrontal lobe and limbic system, playing an important role in regulating the expectation of pain. In this study, acupuncture at bilateral sanyinjiao (SP6) and diji (SP8) points activated both the superior bilateral frontal gyrus and medial frontal gyrus. The primary somatosensory cortex was activated during acupuncture, supporting regulation of acupuncture on the cortex and subcortical brain region. Therefore, these brain regions may be involved in the analgesic effects of acupuncture on dysmenorrhea.

Multiple studies have found that pain stimulation could cause functional activity in the anterior cingulate area, which is associated with pain-induced emotional activity [20-22]. Pain leads to an increase of blood flow of the anterior cingulate cortex, increasing the fMRI signal of this area. However, results of acupuncturing at sanyinjiao (SP6) and diji (SP8) are different. It decreases the signal, indicating that acupuncture analgesia may be related to the regulating effects of the anterior cingulate cortex. Some studies have suggested that basal ganglia (including lenticular nucleus, caudate nucleus, amygdaloid body, and etc.) are involved in deal-
Table 2. Comparing AP and R2 in brain regions with significant differences in ALFF, peak coordinates, and cluster size

<table>
<thead>
<tr>
<th>Location</th>
<th>BA</th>
<th>Peak coordinates (mm)</th>
<th>Peak Z-value</th>
<th>Cluster size (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. extra-nuclear white matter</td>
<td></td>
<td>x 0 y 4 z -4</td>
<td>3.34</td>
<td>330</td>
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<tr>
<td>R. temporal lobe caudate gray matter</td>
<td>40</td>
<td>x 35 y -37 z 2</td>
<td>4.5</td>
<td>135</td>
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<tr>
<td>L. cingulate gyrus</td>
<td>33</td>
<td>x -8 y 20 z 50</td>
<td>4.4</td>
<td>60</td>
</tr>
</tbody>
</table>

Disclosure of conflict of interest

None.

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