The Effectiveness of Transcranial Direct Current Stimulation on the Anxiety and Severity of Stuttering in Adolescents Aged 15 to 18

Mahsa Taherifard, Mohsen Saeidmanesh, Mahdiyeh Azizi

Abstract

Introduction: Since the anxiety is one of the effective factors in stuttering, the present study examined the effect of transcranial direct current stimulation (tDCS) on anxiety and the severity of stuttering in stammering adolescents.

Materials and Methods: The present study adopted pre-test and post-test quasi-experimental design with one control group. For this purpose, after applying inclusion and exclusion criteria, 30 stuttering adolescents were selected by convenience sampling method, and were randomly divided into experimental and control groups. During 10 sessions, one of the experimental groups (EG1) including 15 participants were experimented by 2 mA current of tDCS on two sides of the anterior temporal region for 20 minutes. The other one (EG2) including 15 participants as a sham group experienced tDCS with a similar process for 10 sessions. The difference was that the device was turned off after they felt an initial burning sensation. Anxiety and severity of stuttering were evaluated before and after treatment.

Results: There was a significant difference between the experimental and sham groups in the anxiety of the subjects (P < 0.05, F = 64.725). In addition, there was a significant difference in the severity of stuttering between the experimental and sham groups among participants (P < 0.05, F = 15.897).

Conclusion: According to the results, compared to the sham group, anxiety and the severity of stuttering in stammering adolescents who were affected by tDCS, were reduced significantly. Therefore, it seems that tDCS on both sides of the anterior temporal region can be effective in treating anxiety and severity of stuttering. Though, other similar studies in this field are needed to prove the obtained results.

Keywords: Transcranial direct current stimulation; Stuttering; Anxiety; Temporal area


Introduction

Stuttering is a type of speech disorder that is common to all ages and affects the fluency and temporal pattern of speech (1). Unpleasant experiences that begin as a result of stuttering in childhood are integrated with the physical and social changes that are characteristic to adolescence (2). Since people who stutter are more likely to stutter in the crowd than when they are alone, it is hypothesized that these people are more likely to tolerate anxiety than others. Anxiety, as one of the most well-known emotional problems associated with stuttering, is a response to threatening and challenging stimuli that include various components of cognition and physiological and behavioral responses (3).

In the field of stuttering treatment, speech therapy may improve stuttering by replacing new speech patterns, but creating fluency in these people is not achieved without constant effort and practice, and it is difficult to reach a normal speech level as a result of practicing new conversation patterns, which in turn reduces the acceptability of such methods (4). Moreover, medication and psychotherapy have been commonly used to treat anxiety. At the same time, these two treatments, separately or simultaneously, have been associated with dissatisfaction among patients. Many people still show signs of anxiety after treatment interventions (5). Therefore, it seems that finding other treatments is necessary to improve the...
Treatment outcomes related to anxiety and stuttering.

Transcranial direct current stimulation (tDCS) is a type of non-invasive brain stimulation that modulates neuronal excitability by altering the resting potential of cell membranes. Furthermore, changes in the location of the positive electrode (anode) and negative electrode (cathode) during the tDCS affect the neuronal excitability and responses in different ways (5, 6).

Many neurological disorders are caused by changes in the excitability balance of the brain’s neural circuits. The fact that tDCS can balance excitability and inhibit relatively focal areas of the brain, as well as potentially correct abnormal excitability and abnormal plasticity, makes it clinically attractive (7).

Neuroimaging of the brain of people with stuttering indicates differences in the structure and function of the brain of these people (8). There is a hypothesis that increasing the capacity of the speech motor system as a result of anxiety impairs speech processing. However, due to insufficient research in this field, how anxiety affects stuttering remains unclear (3).

Some studies of functional magnetic resonance imaging (fMRI) show that anxiety disorders are accompanied by abnormal functional activity and connection in the brain (9). Besides, a number of studies have suggested the role of the temporal region on anxiety (10). Meanwhile, Montag et al., in experiments on 110 subjects, reported the importance of white matter in the temporal region on anxiety (11).

Since abnormal alteration of neuroplasticity is an important component of many neurological and psychiatric disorders, non-invasive brain stimulation that is able to modulate neural activity by affecting synaptic flexibility is a potential treatment option (12). There is also a growing interest in using the tDCS therapy to increase neural function and reduce anxiety symptoms (12) and speech disorders (13).

In the first case study, Shiozawa et al. tested tDCS on a 58-year-old woman with generalized anxiety disorder (GAD). In their study, the dorsolateral prefrontal cortex (DLPFC) was cathodically stimulated for 15 consecutive sessions (2 mA); While the anode electrode was located on the opposite side deltoid. They declared the results satisfactory after the intervention and follow-up (30 and 45 days) (14). In their study on 18 patients (46% female and 64% male with a mean age of 28.7 years) with GAD with the protocol proposed by Shiozawa et al., Movahed et al. obtained promising outcomes. Improvement in treatment in their study remained at the follow-up (30 and 45 days) (15). In the field of anxiety disorders, another case study was conducted by Shiozawa et al. on a subject (44 years old female) with panic disorder in which cathodic stimulation (with a current of 2 mA for 30 minutes) was applied to the right DLPFC area in 10 sessions; While the anode electrode was located on the opposite deltoid region. The results indicated a significant reduction in the disease symptoms and these results remained in the follow-up (30 days) (16).

In a double-blind study, Heeren et al. examined 19 female patients with social anxiety disorder (SAD) with anodic stimulation or sham stimulation. The participants underwent anodic stimulation (2 mA for 30 minutes) on the left DLPFC area while completing the probe discrimination task. The findings suggested that anodic stimulation on the left DLPFC region reduced attentional bias in the face of threat (17). Palm et al. conducted the first open-label pilot study using tDCS on 8 patients (mean age 45.6 ± 12.3 years) with phobic postural vertigo (FPV) to modify the disease symptoms (vertigo and dizziness). In this study, the anode electrode (with a current of 2 mA) was placed on the DLPFC area for 5 consecutive days. They found the dizziness-related results to be satisfactory, stating that the improvement in anxiety was not clinically significant (18). Previous studies have investigated the effects of neuronal modulation using tDCS on language processing for healthy and clinical populations (4). In their study, Chesters et al. applied tDCS on the left inferior frontal cortex in 16 participants (mean age 30 years) with stuttering during an anodic stimulation session (2 mA current for 20 minutes). They acknowledged that no significant change was observed in the fluency of speech of the participants (4). In this regard, Chesters et al. conducted another study on 30 adult subjects. Thus, the anode electrode (with a current of 2 mA for 20 minutes) was placed on the left inferior frontal cortex of the subjects for 5 consecutive days. They concluded that tDCS, along with behavioral interventions to induce speech fluency (Behavioral fluency intervention), could increase speech fluency in stuttering adults (13). In a study of 19 participants (4 males with a mean age of 24 years) with the application of tDCS to the Wernicke, Broca, and right homologue, Yada et al. found that cathodic stimulation of the right Broca reduced stuttering (8).

The aim of this study was to evaluate the bilateral effect of tDCS (cathodic and anodic) in the anterior temporal lobe (ATL) on reducing anxiety and stuttering severity in adolescents aged 15 to 18 years with stuttering. As far as the authors of the present study are aware, no other study has been conducted in this field.
Materials and Methods
This study was a quasi-experimental, double-blind study with pretest and posttest design with a control group (sham tDCS). The study population consisted of 15 to 18-year-old adolescents referring to speech therapy centers in Yazd, Iran. The sample size was estimated to be 34 using the Sample power software and statistical power of 0.8 at the alpha level of 0.05. 41 people were enrolled in the study considering the possibility of loss. In terms of ethical considerations, the researcher closely monitored the completion of the questionnaires and explained the right to participate and withdraw from the study, as well as the confidentiality to the participants. The code of ethics in research was received from the University of Science and Arts of Yazd, and finally, people who expressed their consent entered the study. All participants were diagnosed by a master of speech therapy and PhD in clinical psychology using the Stuttering Severity Instrument-4th Edition (SSI-4I) and Beck Anxiety Inventory (BAI) in the clinic of the university. 7 of the subjects were excluded from the study due to lack of the inclusion criteria and 4 ones withdrew from the cooperation in the next stage after the interview and completing the questionnaire. Finally, due to the limited access and since in the experimental studies, the sample size was at least 30 people (19), 30 subjects (17 boys and 13 girls) were randomly divided into two experimental (n = 15) and control (n = 15) groups (Figure 1).

The study inclusion criteria included parents’ and subjects’ consent to participate in the study, stuttering and anxiety, lack of physical and mental disabilities, absence of chronic physical and mental illnesses that could affect the study, lack of simultaneously referring to a speech therapist and a psychologist, and the age range of 15 to 18 years. History of medical or neurological problems such as seizures, brain tumors, post-traumatic brain injury, cardiovascular diseases (CVDs), and problems that increased the risk of using the device and caused complications (burning, itching, etc.) beyond the person’s tolerance due to exposure to tDCS were also considered as the exclusion criteria.

In the present study, the participants were tested three times. In the first stage, the subjects completed the questionnaires and clinical interviews. In the second stage, the participants were randomly divided (by lottery) into two groups of active tDCS (anodic and cathodic, n = 15) and control group (n = 15). The study assistants were trained by a neuroscientist to operate the device (ActivaDose, ActivaTek, USA) and supervised the implementation of the tDCS protocol. In addition, both the assessor and the subjects were unaware of the type of intervention (real or sham tDCS). At this stage, the participants received 10 sessions (5 days a week) of tDCS for 20 minutes each session through a pair of wet and saline sponges (each measuring 35 cm²) on both sides of the ATL. The security of protocols with a current of 1 to 2 mA for 20 minutes has been proven for up to 15 sessions (20). According to the International 10-20 system of electroencephalogram (EEG) lead placement, in all participants, the anode and cathode electrodes were located on the left (T1) and right of the of the ATL (T2), respectively (21). tDCS was performed for the control group with similar conditions. In the control group, after placing the electrodes, the device was turned on and after the initial burning sensation by the patient, it was turned off and the patient did not receive any stimulation for the rest of the time. In all cases, the tDCS was out of sight of the patient. Finally, the subjects were re-evaluated. Moreover, all participants completed their treatment process.

BAI: This questionnaire was designed by Beck and Steer to measure anxiety and consists of 21 items, with four options for each item (not at all, mild, moderate, severe). Each item reflects a symptom of anxiety and is experienced by most people who are clinically anxious or who are in a state of anxiety.
In the BIA scale, the four options of each item are scored in a four-option range from 0 to 3, with the total score ranging from 0 to 63 (22). The validity and reliability of BIA in Iran has been determined by Kaviani and Mousavi (23). In the present study, the construct validity and Cronbach’s alpha coefficient were 0.72 and 0.92, respectively.

SSI-4: This test is a tool to assess the severity of stuttering in individuals with stuttering, which was developed by Riley to objectively and quantitatively measure the severity of stuttering. This tool measures behavioral scales of frequency, duration, and physical behaviors in stuttering. The frequency is scored by the percentage of stuttered syllables and is scored from 2 to 18. The duration scores include an average of three of the longest stuttering events, ranging from 4 to 18. Accompanying physical behaviors are examined in four classes, with each class scoring between 1 and 5. The total score of this class is 0 to 20. The software of this test (CSS, version 20) has been designed to calculate the severity of stuttering and to facilitate the process of scoring of duration and frequency (24). The text of SSI-4 has been translated into Persian by Zolfaghari et al. and its validity and reliability have been confirmed (25).

The obtained data were summarized and described using descriptive statistical methods including frequency calculation, frequency percentage, and table drawing. In order to examine the study variables separately, the performance scores of the groups (experimental and control) in the form of pre-test and post-test stages were analyzed using one-variable analysis of covariance (ANCOVA) test. The normal distribution of the data was evaluated by regression homogeneity test and Kolmogorov-Smirnov (KS) test. The Levene’s test was also used to test the hypothesis of homogeneity of variances. Additionally, multivariate ANCOVA was applied to evaluate the scores of stuttering intensity and anxiety of the groups. Prior to the multivariate ANCOVA, the Box test was used to investigate the homogeneity of the variance-covariance matrices and the Levene’s test was used to test the assumption of homogeneity of variance. Finally, the data were analyzed in SPSS software (version 24, IBM Corporation, Armonk, NY, USA).

**Results**

The most important demographic characteristics of the study groups are presented in table 1.

Then, by calculating the central tendency and dispersion measures, an attempt was made to investigate the distribution and information contained in the study variables related to the effectiveness of tDCS on the ATL on anxiety and stuttering in adolescents aged 15 to 18 years, the results of which are presented in table 2. Accordingly, there were differences between the mean of the experimental and control groups in the pre-test and post-test stages. In order to determine the significance of the changes, the ANCOVA test was employed.

**Table 1. Demographic characteristics of the sample group**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Experimental group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (46.7)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td>Female</td>
<td>8 (53.3)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>7 (46.7)</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>16</td>
<td>3 (20.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>17</td>
<td>4 (26.7)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>18</td>
<td>1 (6.7)</td>
<td>2 (13.3)</td>
</tr>
</tbody>
</table>

After describing the variables and the answers obtained from the statistical population, the study questions were answered using the ANCOVA statistical method.

**Table 2. Comparison of the mean anxiety intervention scores in the pre-test and post-test stages between the experimental and control groups**

<table>
<thead>
<tr>
<th>Sub-scale</th>
<th>Group</th>
<th>Pre-test</th>
<th>Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>Test</td>
<td>31.26 ± 3.23</td>
<td>18.26 ± 2.63</td>
</tr>
<tr>
<td>Stuttering</td>
<td>Control</td>
<td>29.73 ± 5.13</td>
<td>29.46 ±5.18</td>
</tr>
<tr>
<td>intensity</td>
<td>Test</td>
<td>15.20 ± 1.32</td>
<td>7.46 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>15.46 ± 1.55</td>
<td>15.60 ± 1.45</td>
</tr>
</tbody>
</table>

Data are reported as mean ± standard deviation (SD).

In order to investigate the anxiety in the subjects, the level of scores of the executive functions of the groups (control and test) in the pre-test and post-test stages were analyzed using one-variable ANCOVA. It should be noted that before performing the ANCOVA, its assumptions were examined.

The results of the homogeneity tests of regression slope ($P > 0.050$, $F = 1.832$) and KS test to investigate the assumption of normal distribution ($P > 0.050$, $F = 2.540$), along with the hypothesis of homogeneity of variances using the Levene’s test ($P > 0.050$, $F = 0.808$) for anxiety score showed that the level of significance in these tests was more than 0.05. Therefore, the one variable ANCOVA could be applied.

Based on the data presented in table 3, considering the pre-test scores of anxiety as a covariate (auxiliary) variable, the difference between the experimental and control groups was significant ($P < 0.050$, $F = 64.725$).
In other words, it can be said that the difference between the scores of the two groups indicated that tDCS in the ATL was effective on anxiety in adolescents aged 15 to 18 years and the effect level rate was 1,000.

In order to evaluate the severity of stuttering in the subjects, the scores of the executive functions of the subjects in the two groups (control and test) in the pre-test and post-test stages were analyzed using single-variable ANCOVA. Before performing ANCOVA, its hypotheses were tested. The results of the homogeneity tests of regression slope (P > 0.05, F = 3.216) and KS test to investigate the assumption of normal distribution (P > 0.05, F = 3.32), along with the hypothesis of homogeneity of variances using the Levene’s test (P > 0.05, F = 0.860), for the stuttering intensity score revealed that the significance level in these tests was more than 0.05. Therefore, there was no problem in using the single-variable ANCOVA.

The data in table 4 indicated that considering the pre-test scores of stuttering intensity as a covariate variable (auxiliary), the difference between the experimental and control groups (P < 0.050, F = 15.897) was significant. In other words, it can be said that the difference between the scores of the two groups of adolescents indicated that tDCS in the ATL was effective on the stuttering of adolescents aged 15 to 18 years and the effect level was 0.97.

Furthermore, multivariate ANCOVA was applied to evaluate the level of stuttering intensity and anxiety scores of the groups. It should be noted that before performing multivariate ANCOVA, its assumptions were examined. The results of the Box test to investigate the homogeneity of variance-covariance matrices (P > 0.05, F = 2.730) and the Levene’s test results to investigate the assumption of homogeneity of variances in stuttering intensity (P > 0.05, F = 0.878) and anxiety (P > 0.05, F = 1.186) showed that the level of significance in these variables was more than 0.05. Therefore, there was no problem in using the multivariate ANCOVA (Table 5).

### Table 4. Results of single-variable analysis of covariance (ANCOVA) to compare the stuttering scores of the control and experimental groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean squares</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity of stuttering</td>
<td>Pre-test</td>
<td>15.897</td>
<td>1</td>
<td>15.897</td>
<td>15.897</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>474.909</td>
<td>1</td>
<td>474.909</td>
<td>474.909</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>25.436</td>
<td>27</td>
<td>9.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4528</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant at the level of P ≤ 0.05

### Table 3. Results of single-variable analysis of covariance (ANCOVA) to compare the anxiety scores of the control and experimental groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean squares</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety</td>
<td>Pre-test</td>
<td>333.534</td>
<td>1</td>
<td>333.534</td>
<td>64.725</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>1121.014</td>
<td>1</td>
<td>1121.014</td>
<td>217.543</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
<td>139.133</td>
<td>27</td>
<td>5.153</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>18502</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the level of P ≤ 0.05

### Discussion

In the present double-blind experimental study, 30 adolescents aged 15 to 18 years with stuttering were randomly divided into the two experimental and control groups to receive 2 mA tDCS on both sides of the ATL during 10 daily sessions. The findings suggested that tDCS on both sides of the ATL in the post-test phase improved anxiety and severity of stuttering in the adolescents. Moreover, tDCS in the control group had a much greater effect on improving anxiety and stuttering intensity compared to the control group. The effect of tDCS on improving anxiety in adolescents aged 15 to 18 years with stuttering supports the hypothesis that the temporal lobe is involved in anxiety (11). Findings from the present study on anxiety were consistent with the results of the study by Montag et al. on the effect of white matter abnormality in the temporal lobe on anxiety (11). There are several possible explanations for the results of the present study. Many studies have shown the major effect of the temporal/hippocampal region on negative emotions.

### Table 5. Results of significance test of multivariate analysis of covariance (ANCOVA) on the severity of stuttering and anxiety in the experimental and control groups

<table>
<thead>
<tr>
<th>Wilks’s lambda</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>0.037</td>
<td>326.088</td>
<td>2</td>
<td>25</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Significant at the level of P ≤ 0.05
df: Degree of freedom

In the present double-blind experimental study, 30 adolescents aged 15 to 18 years with stuttering were randomly divided into the two experimental and control groups to receive 2 mA tDCS on both sides of the ATL during 10 daily sessions. The findings suggested that tDCS on both sides of the ATL in the post-test phase improved anxiety and severity of stuttering in the adolescents. Moreover, tDCS in the control group had a much greater effect on improving anxiety and stuttering intensity compared to the control group. The effect of tDCS on improving anxiety in adolescents aged 15 to 18 years with stuttering supports the hypothesis that the temporal lobe is involved in anxiety (11). Findings from the present study on anxiety were consistent with the results of the study by Montag et al. on the effect of white matter abnormality in the temporal lobe on anxiety (11). There are several possible explanations for the results of the present study. Many studies have shown the major effect of the temporal/hippocampal region on negative emotions.
Furthermore, in individuals with high anxiety, Yang et al. observed significant abnormalities in the white matter that connects the temporal lobe/hippocampus with other areas of the brain (10). In addition, there is evidence of unbalanced performance within and among frontolimbic systems, which appears to be due in part to microstructural changes in the Uncinate fasciculus white matter categories and cingulum that connect the key nodes of these systems. Uncinate fasciculus is a group of white matter in the human brain that connects parts of the limbic system in the ATL, such as the hippocampus and amygdala, to the frontal parts, such as the orbitofrontal cortex (26).

Given the findings of the present study, abnormalities in the function of this area play an important role in stuttering (27), trait anxiety (10), and GAD (28). On the basis of the results of previous studies, the effect of tDCS varies depending on the polarization of the electrode, with the anodic and cathodic polarization increasing and decreasing cortical excitability, respectively (2). Probably one of the reasons for the decrease in stuttering intensity in the subjects was the placement of the cathode on the right side of their brains and the modulation of the function of their right hemisphere. In this regard, the findings of the present study can support the results of previous studies on the existence of hyperactivity in the right side of the brain of people with stuttering (29). In addition, the results of the present study can strengthen the hypothesis proposed by Neef et al. on the effectiveness of Uncinate fasciculus on stuttering (27). On the other hand, the basal mechanism of tDCS seems to be related to some major physiological effects such as subthreshold polarization of nerve membranes as well as factors affecting neurotransmitters such as serotonin reuptake, Y-aminobutyric acid (GABA), glutamatous neuronal firing, and dopamine (30). This modulatory property of tDCS can help improve the function of the white matter structure in the ATL and anxiety-related structures such as the Uncinate fasciculus, cingulum, and temporal/hippocampal areas (26) in the brain of people with stuttering, thereby reducing their anxiety. Therefore, the present study can also support the hypothesis that Uncinate fasciculus is involved in anxiety (28). Besides, given the fact that the function of the ATL, and especially the Uncinate fasciculus, is important in anxiety and stuttering, it remains to be seen whether reduced anxiety reduces stuttering.

Limitations

Limitations of the present study included low tDCS concentricity and simultaneous effect on the action of other areas of the brain related to the stimulation area, limited age range, lack of follow-up of long-term effects of the intervention, and no use of neuroimaging.

Recommendations

Other researchers are recommended to consider post-intervention follow-up measures in future neuroimaging studies to evaluate long-term effects and to conduct similar studies on different ages.

Conclusion

Taking into account the results of the present study, it seems that tDCS on both sides of the ATL can be effective in improving anxiety and severity of stuttering in adolescents aged 15 to 18 years. The findings suggested that active tDCS was much more effective than sham stimulation. Additionally, there was no obvious difference in the control group and the pre-test results. Definitely more evidence is needed to confirm the present findings.

Acknowledgments

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Authors’ Contribution

Mahsa Taherifard: study design and ideation, study support, executive, and scientific services, providing study equipment and samples, data collection, analysis and interpretation of results, specialized statistics services, manuscript preparation, specialized manuscript evaluation in terms of scientific concepts, final manuscript approval for submission to the journal office, responsibility of maintaining the integrity of the study process from the beginning to the publication, and responding to the referees’ comments; Mohsen Saeidmanesh: study design and idea, attracting financial resources for the study, study support, executive, and scientific services, providing study equipment and samples, data collection, analysis and interpretation of results, manuscript preparation, specialized manuscript evaluation in terms of scientific concepts, final manuscript approval for submission to the journal office, responsibility of maintaining the integrity of the study process from the beginning to the publication, and responding to the referees’ comments; Mahdiyeh Azizi: study support, executive, and scientific services, analysis and interpretation of results, specialized
manuscript evaluation in terms of scientific concepts, final manuscript approval for submission to the journal office, responsibility of maintaining the integrity of the study process from the beginning to the publication, and responding to the referees’ comments.

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**Conflict of Interest**

Authors do not have a conflict of interest.

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