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Research paper

Comparative effectiveness of two different doses of botulinum toxin A for the treatment of mild to moderate depression

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ABSTRACT

Objective: Botulinum toxin A has been shown to be effective in managing depression. This study aimed to evaluate the antidepressant and antianxiety effects of two different doses of botulinum toxin A in patients with mild to moderate depression.

Methods: A total of 140 patients diagnosed with mild to moderate depression at the Department of Neurology of the Second Affiliated Hospital of Soochow University from September 2020 to September 2021 were enrolled for the study. The patients were allocated into two groups and treated with two different doses of botulinum toxin A (50 units or 100 units). Depression scores (HAMD, HAMA, SDS, and SAS) were evaluated at baseline and 1, 2, 4, 8, and 12 weeks after treatment.

Results: There was a significant improvement in the depressive and anxiety symptoms following treatment with the botulinum toxin A after 12 weeks compared to the baseline. However, there were no significant differences between the two groups. Further, the factor scores of anxiety/somatization, blocking, sleep disorder, and cognitive disorder were significantly decreased after 12 weeks of treatment with 50 units of botulinum toxin A compared to the baseline ($P < 0.05$). Further, the factor scores of somatic and mental anxiety were significantly decreased at different time points after treatment with 50 units of botulinum toxin A compared to the baseline ($P < 0.05$).

Conclusion: Local injections of 50 units and 100 units of botulinum toxin A shows equal efficacy. Therefore, 50 units of botulinum toxin A could be used clinically to manage mild to moderate depression.

1. Introduction

Depression is the most common mental health disorder (Kessler et al., 1994; Sinyor et al., 2016), characterized by sadness, loss of interest, pleasure or appetite, feelings of guilt, low self-worth or tiredness, disturbed sleep, and poor concentration (Lu et al., 2014). According to the World Health Organization, depression affected >264 million people worldwide in January 2020 (Disease et al., 2018). Depression can be classified into mild, moderate, or severe forms of the disease. Mild to moderate depression is highly prevalent and is often accompanied by anxiety. It is commonly underdiagnosed and undertreated due to the subjective nature of the symptoms (Bess et al., 2013). Increased rates of screening would increase the detection and treatment of depression (Samples et al., 2020). Depression and anxiety disorders are common comorbidities. For example, the Netherlands Study of Depression and Anxiety (NESDA) reported that of those patients with a primary anxiety disorder diagnosis, 63 % were diagnosed with current, while 81 % were diagnosed with a lifetime depressive disorder (Groen et al., 2020).

Depression can be managed using pharmacological treatment and/or psychological therapy. However, treatment response is often unsatisfactory. Consequently, depression may become chronic in a considerable proportion of patients. Therefore, there is a need to develop effective therapies to improve the course and prognosis of depressive disorders. Furthermore, researchers need to develop alternative approaches for preventing and treating depression.

Recent evidence revealed that botulinum toxin A (BoNT/A) was an effective therapy for improving the symptoms of depression. A previous study reported positive mood effects in patients treated for glabellar frown lines with BoNT/A. Furthermore, the first randomized controlled trial investigation showed an antidepressant effect of BoNT/A (Wollmer

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et al., 2012). In addition, a previous study showed that peripheral facial injection of BoNT/A was rapid, effective, and relative safe for improving some symptoms of depression. Moreover, a Randomized Controlled Trial (RCT), demonstrated that BoNT/A could be used as an effective antidepressant (Li et al., 2021).

BoNT/A is a metalloprotease that targets and cleaves synaptosomal-associated protein of 25 kDa (SNAP-25), a member of the SNARE (soluble NSF-attachment protein receptor) family. It blocks the release of neurotransmitters such as acetylcholine from the peripheral nerve terminals. Local injections of BoNT/A cause cleavage of SNAP-25 at the neuromuscular junction, leading to muscle relaxation. SNAP-25 is critical in the fusion of the synaptic vesicle to the inner surface of the cellular membrane (Rossetto et al., 2014). However, the precise mechanism underlying how BoNT/A alleviates symptoms of depression has not been elucidated. Nonetheless, it is assumed that the reduced proprioceptive feedback from the paralyzed facial muscles could be a relevant mechanism of mood improvement. This mechanism is supported by some studies that showed that treatment with botulinum toxin was associated with positive effects on emotional perception (Havas et al., 2010; Hennenlotter et al., 2009). Other studies revealed that retrograde transport could be behind BoNT/A, relieving the symptoms of depression (Cai et al., 2017; Mazzocchio and Caleo, 2015). Further, a previous study showed increased brain-derived neurotrophic factor in the hippocampus and activation of the downstream ERK-CREB signaling pathways following local injection of BoNT/A in stressed-mice (Li et al., 2019).

Previous studies showed that a single injection of BoNT/A into facial muscles in the glabellar region could be used as a novel, well-tolerated treatment option for depression (Finzi and Wasserman, 2006; Magid et al., 2014; Wollmer et al., 2012). In addition, the antidepressant effects of BoNT/A were shown to last for several months following a single treatment session. However, the studies used different doses of BoNT/A as there is no consensus on the dose of BoNT/A for the management of depression. Therefore, it is necessary to explore the appropriate dose of BoNT/A for the management of depression. This study explored two different doses of BoNT/A in patients with mild to moderate depression.

2. Materials and methods

2.1. The study population

This was a randomized prospective study evaluating the efficacy of BoNT/A (Hengli, Cat. No. S10970037, origin: Lanzhou, China) in managing mild to moderate depression. A total of 140 patients who were diagnosed with mild to moderate depression at The Second Affiliated Hospital of Soochow University from September 2020 to September 2021 were enrolled. The patients had mild to moderate depressive episodes lasting ≥2 weeks. The participants were randomly assigned to one of two treatment groups, each having 70 patients, who received BoNT/A injections of either 100 units (U) or 50 units (U). The basic information of the participants, including age, gender, race, and education level, was recorded. All patients signed informed consent before participating in the study.

The inclusion criteria were:

(1) Meeting the DSM-IV criteria which comprise for minor to major depression, patients with anxiety disorders or comorbid anxiety disorders were not included;
(2) Aged 18–75 years;
(3) A score on the 17 Items Hamilton Depression Scale (HAMD) (Hamilton, 1960) of 7 ≤ HAMD-17 ≤ 24;
(4) right-handed:
(5) emmetropia.

The exclusion criteria were:

(1) patients with other psychiatric disorders other than depression;
(2) patients with other systemic diseases and organic brain diseases caused by depression;
(3) patients with a previous history of head disease, brain injury, epilepsy, or other nervous system diseases;
(4) patients with a previous history of severe cardiovascular diseases, respiratory diseases, or diseases of the immune system;
(5) Patients with a use history of antipsychotics, antidepressants, sedatives, alcohol, morphine, or drug use disorders;
(6) patients with liver or kidney function impairment;
(7) Patients who refused to complete the relevant neuropsychological tests;
(8) pregnant and lactating women.

2.2. Drug administration

A total of 100 U or 50 U of BoNT/A were diluted with 0.9 % saline to make a final volume of 2 ml. The injection scheme was adopted according to previous research (Brin et al., 2009) and our own studies (Li et al., 2022; Zhang et al., 2021). The dose was injected into ten sites simultaneously, including the glabellar region muscle, depressor supercilii muscle, and occipital frontalis muscle. Further, the drug was injected into ten more sites, including at the lateral canthus of the eyes and the bilateral temporal region. In total, 20 sites were injected with 5 U or 2.5 U per site (Fig. 1). The reconstituted drug that was not administered immediately was stored at 2–8 °C and used within 4 h.

2.3. Assessments

After randomization and treatment, all patients were followed up in the outpatient clinic for 12 weeks. Depression was assessed using the HAMD-17, 14-item Hamilton Anxiety Scale (HAMA), Self-rating Depression Scale (SDS), and Self-rating Anxiety Scale (SAS) at 1,2,4,8 and 12 weeks. Patients who required concomitant treatment for depression or a change in the cognitive therapy regimen were discontinued from the study. Further, the patients were observed for any

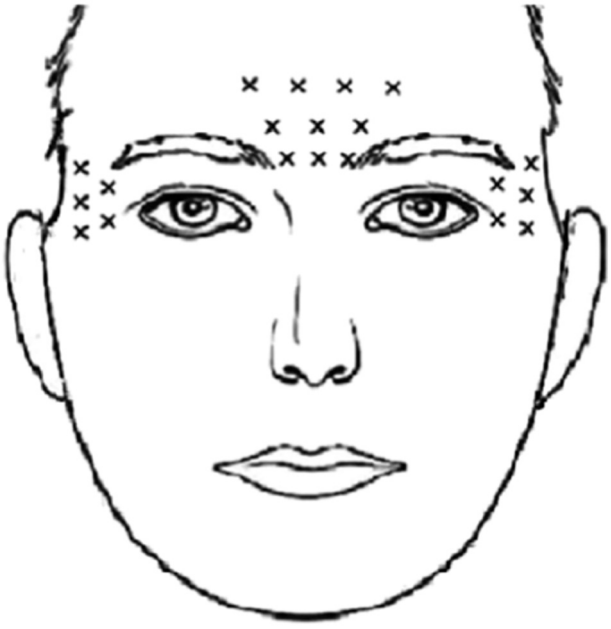

Fig. 1. Schematic representation of BoNT/A injection sites. (X stands for the injection site, 10 points for glabellar region muscle, depressor supercilii muscle, and occipital frontalis muscle, five points each for lateral canthus, and bilateral temporal region. A total of 20 sites and 5 units or 2.5 units per site were applied for the BoNT/A.)

allergic reactions for the first 30mins of treatment administration. In addition, constant communication was maintained.

2.4. Ethical approval

The study protocols were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Suzhou University (number: JD-LK-2017-011-02). Further, the study was registered under the Chinese Clinical Trial Registry (ChiCTR1800019802). All patients signed written informed consent.

2.5. Statistical analysis

Data were presented using mean ± S.E.M. Statistical analyses were performed using the Graphpad Prism 8.0 statistical software. The HAMD, HAMA, SDS, and SAS scores obtained before and after treatment were compared using the Two-way repeated-measure ANOVA following Bonferroni post hoc correction. Differences between the two groups were determined using an unpaired *t*-test. One-way ANOVA following Bonferroni post hoc correction was used to compare scores at different follow-up time points. *P*-values<0.05 were considered statistically significant.

3. Results

3.1. Baseline characteristics

The study population consisted of 140 patients who were randomly allocated into two groups ($n = 70$). Only 122 patients completed 12 weeks of follow-up. However, 18 patients were lost-to-follow-up due to the following reasons: five patients (100 U BoNT/A group ($n = 2$; 2.9 %) and 50 U BoNT/A group ($n = 3$; 4.3 %) were withdrawn from the study as they were reluctant to follow the doctor's orders,seven patients (100 U BoNT/A group ($n = 4$; 5.7 %) and 50 U BoNT/A group (n = 3; 4.3 %)) actively withdrew from the study as they did not see any significant improvement with the study drug, while six patients (100 U BoNT/A group (n = 3; 4.3 %) and 50 U BoNT/A group (n = 3; 4.3 %)) were not completely followed-up due to the COVID-19 epidemic or personal reasons. The baseline characteristics of the patients are summarized in Table 1.

3.2. Determination of the antidepressant and antianxiety effects

3.2.1. Treatment with 100 U BoNT/A shows antidepressant and antianxiety effects

The HAMD scores were shown to be decreased after follow-up for 12 weeks compared with the baseline scores (Fig. 2a. F (5, 299) = 37.83, $P < 0.0001$). Similarly, the SDS scores were decreased at 12 weeks follow-up compared to the baseline (Fig. 2c. F (5, 298) = 4.859, $P = 0.0003$). Furthermore, the mean of HAMD and SDS scores assessed at 12 weeks and baseline was shown to decrease from 12.46 ± 0.56 to 6.57 ± 0.48 and 42.08 ± 1.13 to 32.52 ± 0.84, respectively. In addition, 100 U BoNT/A was shown to significantly decrease the HAMA scores at 12 weeks follow-up compared to the baseline (Fig. 2b. F (5, 300) = 26.96, $P < 0.0001$). Similarly, the SAS scores were shown to be decreased at 12 weeks follow-up compared to the baseline (Fig. 2d. F (5, 300) = 33.00, P < 0.0001). Moreover, the mean of HAMA and SAS scores determined after 12 weeks and at baseline were shown to decrease from 12.44 ± 0.82 to 6.69 ± 0.48 and 42.21 ± 1.09 to 33.26 ± 0.79, respectively. Taken together, these results suggest that 100 U BoNT/A shows antidepressant and antianxiety effects.

Table 1
Baseline characteristics of participants. Abbreviations: BoNT/A, botulinum toxin A; HAMA, Hamilton Depression Scale; HAMD, Hamilton Depression Scale; SAS, Self-Rating Anxiety Scale; SDS, Self- Rating Depression Scale.

	100 U ($n = 61$)	50 U (n = 61)	*p* value	Upaired t-test
Age (Year)	63.41 ± 1.37	60.49 ± 2.08	0.243	
Gender (female, %)	41 (45.56 %)	49 (54.44 %)	0.158	
Dropped out	9 (12.86 %)	9 (12.86 %)	0.999	
HAMD	12.46 ± 0.56	11.85 ± 0.57	0.449	0.760
HAMA	12.44 ± 0.82	12.79 ± 0.59	0.733	0.342
SDS	42.08 ± 1.13	41.11 ± 1.04	0.527	0.634
SAS	42.21 ± 1.09	40.16 ± 0.76	0.126	1.540

3.2.2. Treatment with 50 U BoNT/A shows antidepressant and antianxiety effects

The HAMD scores were shown to be decreased at 12 weeks follow-up compared to the baseline (Fig. 2a. F (5, 300) = 38.11, $P < 0.0001$). Similarly, the SDS scores were shown to be decreased at 12 weeks follow-up compared to the baseline (Fig. 2c. F (5, 300) = 47.68, P < 0.0001). Furthermore, the mean of HAMD and SDS scores assessed at 12 weeks and baseline was shown to decrease from 11.85 ± 0.57 to 6.23 ± 0.50 and 41.11 ± 1.04 to 30.82 ± 0.73, respectively. In addition, the HAMA scores were decreased at 12 weeks follow-up compared to the baseline (Fig. 2b. F (5, 300) = 56.92, P < 0.0001). Similarly, the SAS scores were decreased at 12 weeks follow-up compared to the baseline (Fig. 2d. F (5, 300) = 44.32, P < 0.0001). Moreover, the mean of HAMA and SAS scores determined after 12 weeks and at baseline was shown to decrease from 12.79 ± 0.59 to 6.03 ± 0.54 and 40.16 ± 0.76 to 30.66 ± 0.57, respectively. Taken together, these results suggest that 50 U BoNT/A shows antidepressant and antianxiety effects.

3.2.3. Treatment with 50 U or 100 U BoNT/A shows similar antidepressant and antianxiety effects

Decreased HAMD scores were observed in the two groups at 1, 2, 4, 8, and 12 weeks. However, there were no significant differences in HAMD scores at different time points between the two groups (Fig. 3a. $t_1 = 0.581$, $P > 0.05$; $t_2 = 0.754$, $P > 0.05$; $t_4 = 1.005$, $P > 0.05$; $t_8 = 0.377$, $P > 0.05$; $t_{12} = 0.440$, $P > 0.05$). Similarly, there were no significant differences in SDS scores at different time points between the two groups (Fig. 3b. $t_1 = 0.548$, $P > 0.05$; $t_2 = 1.402$, $P > 0.05$; $t_4 = 1.401$, $P > 0.05$; $t_8 = 1.974$, $P > 0.05$; $t_{12} = 0.913$, $P > 0.05$). Further, there were no significant differences in HAMA scores at different time points between the two groups (Fig. 3c. $t_1 = 0.252$, $P > 0.05$; $t_2 = 0.775$, $P > 0.05$; $t_4 = 0.872$, $P > 0.05$; $t_8 = 0.330$, $P > 0.05$; $t_{12} = 0.775$, $P > 0.05$). In addition, there were no significant differences in SAS scores at different time points between the two groups (Fig. 3d. $t_1 = 2.043$, $P > 0.05$; $t_2 = 1.987$, $P > 0.05$; $t_4 = 1.667$, $P > 0.05$; $t_8 = 0.572$, $P > 0.05$; $t_{12} = 2.261$, $P > 0.05$). Moreover, the mean HAMD scores at 12 weeks follow-up in the 100 U and 50 U BoNT/A groups were 6.574 ± 0.483 and 6.230 ± 0.502, respectively. In contrast, the mean SDS, HAMA, and SAS scores at 12 weeks in the 100 U BoNT/A group were 32.52 ± 0.844, 6.689 ± 0.483, and 33.26 ± 0.792, respectively. However, the mean SDS, HAMA and SAS scores at 12 weeks in the 50 U BoNT/A group were 30.82 ± 1.042, 6.033 ± 0.537 and 30.66 ± 0.569, respectively. The results showed no significant differences between the two groups. Taken together, the results revealed that local injections of 50 U and 100 U BoNT/A had equal efficacy. As there are some patients dropped out of the study, The findings presented in the manuscript are the results of a per protocol set analysis. We find there was no any significant difference between an intention to treat and per protocol analysis of the study data.

3.2.4. Treatment with 50 U BoNT/A decreased the factor scores for anxiety

The HAMD item scores were divided into five parts for factor scoring, including anxiety/somatization, blocking, sleep disorder, cognitive disorder, and weight loss. The HAMD scores in anxiety/somatization, blocking, sleep, and cognitive disorders were shown to be significantly decreased after treatment with 50 U BoNT/A for 12 weeks. The HAMD score in anxiety/somatization was significantly decreased at 12 weeks compared with the baseline (Fig. 4a. F (5, 300) = 17.02, $P < 0.0001$). Similarly, the HAMD score in blocking was significantly decreased at 12

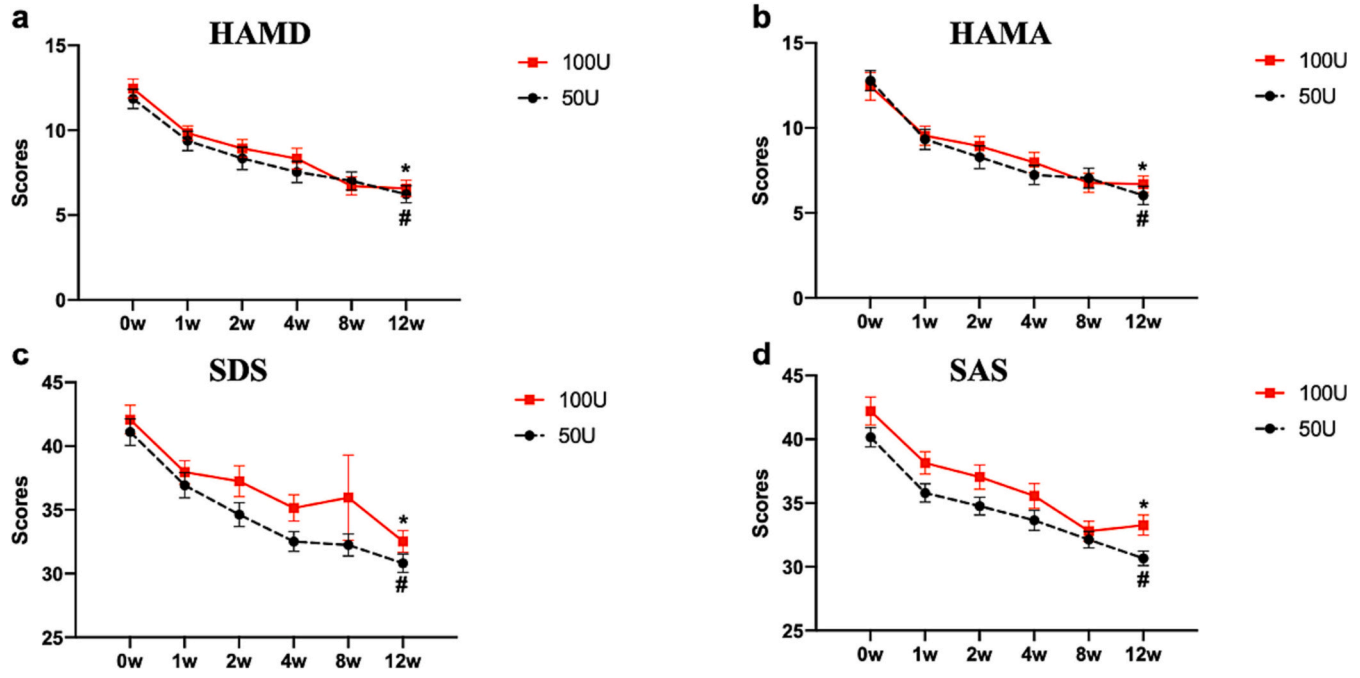

Fig. 2. 100 U and 50 U BoNT/A decreased the scores of HAMD, HAMA, SDS, and SAS after 12 weeks of therapy when comparing with baseline (a, HAMD; b, HAMA; c, SDS; d, SAS). The scores of HAMD, HAMA, SDS, and SAS of 100 U and 50 U groups were determined using two-way repeated-measured ANOVA (#, *: there was statistical significance between scores of 12 weeks and baseline, $p < 0.05$, # for the 50 U group, * for the 100 U group).

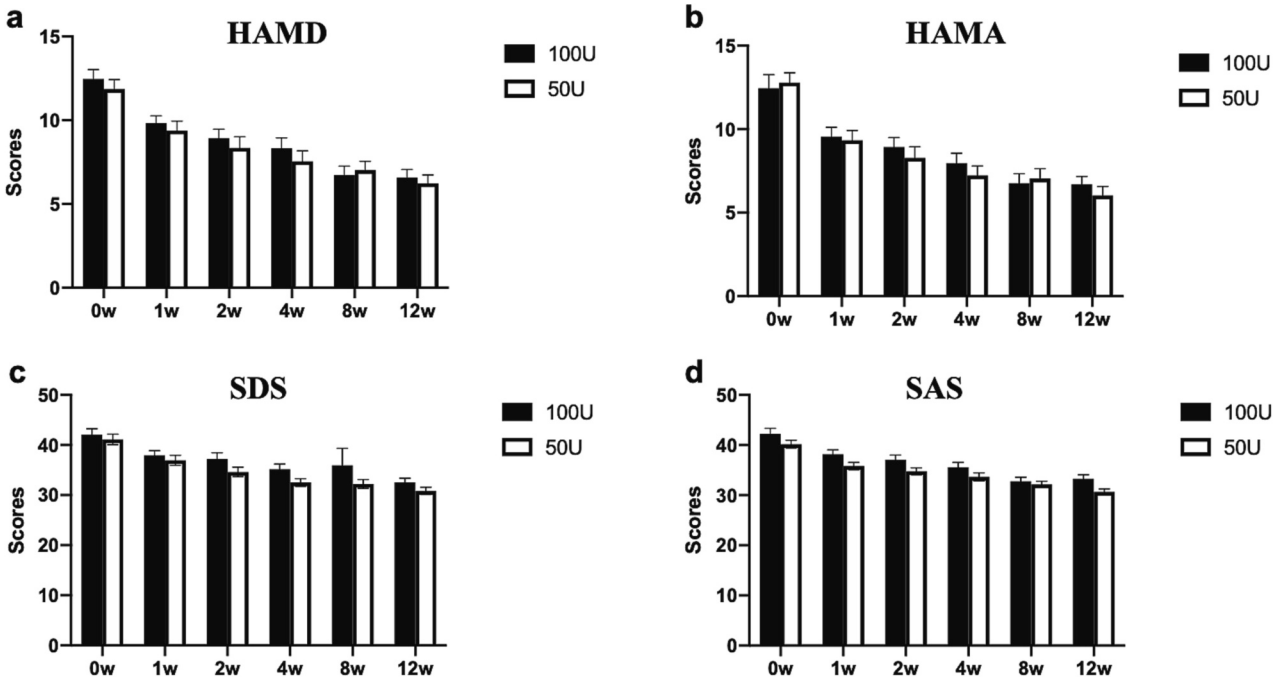

Fig. 3. The difference between 100 U and 50 U BoNT/A groups at each time point was determined using the unpaired *t*-test. There were no statistical significance between 100 U and 50 U at different time points.

weeks compared with the baseline (Fig. 4a. F (5, 300) = 33.78, P < 0.0001). In addition, the HAMD score in sleep disorder (Fig. 4a. F (5, 299) = 10.76, P < 0.0001) and cognitive disorder (Fig. 4a. F (5, 300) = 13.49, P < 0.0001) were significantly decreased at 12 weeks compared with the baseline. However, the HAMD score in weight loss did not show a significant decrease at 12 weeks compared to the baseline (Fig. 4a. F (5, 300) = 2.117, $P = 0.063$).

The anxiety symptoms were divided into somatic anxiety and mental anxiety item scores. The results showed significantly decreased somatic anxiety and mental anxiety factor scores at the different time points following treatment with 50 U BoNT/A. Furthermore, the HAMA scores of somatic anxiety were shown to be decreased at 12 weeks compared to the baseline (Fig. 4b. F (5, 299) = 35.45, P < 0.0001). Similarly, the factor scoring for mental anxiety showed a significant decrease at 12 weeks compared to the baseline (Fig. 4b. F (5, 299) = 37.63, P < 0.0001). Taken together, the decreased factor scores suggest that

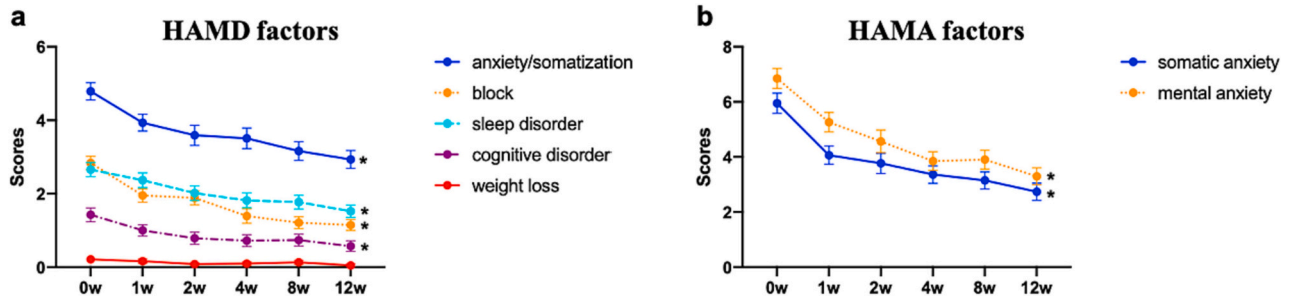

Fig. 4. 50 U BoNT/A decreased the factor scores of HAMD and HAMA after 12 weeks of therapy when comparing with baseline (a, HAMD factors; b, HAMA factors). The scores of HAMD factors and HAMA factors were determined using two-way repeated-measured ANOVA. *: there was statistical significance between the factor scores of 12 weeks and baseline, $p < 0.05$.

treatment with 50 U BoNT/A has antidepressant and antianxiety effects.

3.3. Adverse events

The observed adverse events were summarized in Table 2. The patients were observed for at least half an hour after the local injection of BoNT/A. There were no acute side effects observed. In the 100 U BoNT/A group comprising of 61 patients, 6 (9.84 %) showed brow muscles stiffness, 2 (3.28 %) had bilateral eyebrow asymmetry, 2 (3.28 %) had both brow muscles stiffness and bilateral eyebrow asymmetry, while 2 (3.28 %) had eyelid ptosis. On the other hand, the 50 U BoNT/A group comprising of 61 patients, 4 (6.56 %) showed brow muscles stiffness, 2 (3.28 %) had bilateral eyebrow asymmetry, 1 (3.28 %) had both brow muscles stiffness and bilateral eyebrow asymmetry, while no patient had eyelid ptosis. The symptoms completely disappeared within six weeks of treatment. Adverse events related to other systems, such as gastrointestinal effects or headaches, were not reported.

4. Discussion

Patients in the two groups showed a significant improvement at 12 weeks of follow-up. Further, there were no significant changes between the two groups at different time points of follow-up. In addition, the two doses showed equal efficacy and a similar short-term safety profile. Moreover, the HAMD and HAMA scores were decreased to <7 in the two treatment groups at 12 weeks. And patients' personal mood experiences after treatment was more individualized than that in SDS and SAS scores. Moreover, facial injection of 50 U BoNT/A decreased the HAMD factor scores in anxiety/somatization, blocking, sleep disorder and cognitive disorder. However, the HAMD factor score in weight loss was not decreased. Some patients reported adverse effects following treatment with BoNT/A. However, there were no significant differences in the adverse effects reported between the two groups. Therefore, a low dose of BoNT/A injections should be employed in clinical practice to relieve depression and anxiety symptoms.

Increasing evidence shows that BoNT/A plays a significant role in managing depression. Recently, various reports showed the beneficial role of BoNT/A in managing depression. Finzi and Wasserman, 2006 reported that facial injection with 29 U of BoNT/A showed antidepressant effects (Finzi and Wasserman, 2006). Moreover, Hexsel et al. reported that peak effects of BoNT/A were reported after one month following facial injection with 20 U BoNT/A and the effects lasted for three to six months. Furthermore, the maximum effects were reported within the first two months of treatment (Hexsel et al., 2013b). Moreover, Wollmer et al. demonstrated that a single treatment session of injecting BoNT/A (39 U in men and 29 U in women) into the glabellar region reduced the symptoms of major depression. The effects were observed within a few weeks and persisted throughout the sixteen weeks of follow-up (Wollmer et al., 2012). Further, a study conducted by Magid et al. showed a statistically significantly decrease in depressive symptoms in patients who received BoNT/A (39 U in men and 29 U in women) injections into the glabellar region muscles. The effects were observed over the 24-weeks of follow-up (Cheng et al., 2018). In addition, Finzi and Rosenthal showed that a single treatment session with injections of BoNT/A, (40 U in men and 29 U in women), to the corrugator and procerus muscles induced significant and sustained antidepressant effects (Finzi and Rosenthal, 2014). Further, previous studies suggested that injecting the glabellar region muscles of depressed patients with BoNT/A improved the symptoms of depression (Finzi and Wasserman, 2006; Wollmer et al., 2012). However, the underlying mechanism of action remains unclear. These studies were conducted in patients with major depression. However, to the best of our knowledge, no study has evaluated the antidepressant effects of BoNT/A in patients with mild to moderate depression in Chinese population settings. The present study demonstrated that BoNT/A injection in mild to moderate depression was efficacious. Therefore, injections of BoNT/A could be clinically utilized to manage mild to mild depression.

Several theories have been postulated to explain how BoNT/A injections improve mood (Alam et al., 2008). One suggestion is that the mere cosmetic effect of the procedure makes people feel better about themselves. Recent imaging studies showed that imitation of facial expressions was associated with activation in the limbic regions such as the amygdala. However, the physiological interaction between limbic activation and facial feedback has not been elucidated (Hennenlotter et al., 2009). Another theory suggests that people who look happier are received more favorably by those around them, leading to positive social feedback and in turn, a happier mood. Further, evidence suggests that facial expressions play a causal role in the emotional experience (Adelmann and Zajonc, 1989).

The neuromuscular junction is the natural target of BoNT/A. Following injection into a muscle, BoNT/A is internalized by motoneuron terminals. It functions as an endopeptidase, which cleaves protein components of the synaptic machinery responsible for vesicle docking and exocytosis. As a result, BoNT/A induces a characteristic flaccid paralysis of the affected muscle. Moreover, treatment with BoNT/A improve the quality of life and satisfaction (Hexsel et al., 2013a), reduced fear and sadness (Lewis and Bowler, 2009), and increased emotional well-being beyond the cosmetic effects (Sommer

Table 2
Side effects occurred in the 100 U and 50 U groups received BoNT/A treatment.

	100 U (n = 61)	50 U (n = 61)	*p* value
Brow muscles stiffness	6 (9.84 %)	4 (6.56 %)	
Bilateral eyebrow asymmetry	2 (3.28 %)	2 (3.28 %)	
Both Brow muscles stiffness and Bilateral eyebrow asymmetry	2 (3.28 %)	1 (3.28 %)	
Eyelid ptosis	2 (3.28 %)	0 (0.00 %)	
No side effects	49 (80.33 %)	54 (88.52 %)	0.212

et al., 2003). Treatment with BoNT/A has a well-tolerable safety profile associated with minimal side effects as it is injected locally into the corrugator and procerus muscles of the forehead (Brin et al., 2009). Brin et al. shows two different doses of onabotulinumtoxinA for the treatment of major depressive disorder (Brin et al., 2020). In addition, a single dose of BoNT/A has long-term effects, which ensures better adherence and is cost-effective due to the longer administration drug intervals. Further, BoNT/A has fewer drug-drug interactions than other drugs used for managing depression.

In conclusion, this study shows that a single injection of 100 U or 50 U BoNT/A can be employed as a safe and effective treatment for mild to moderate depression. In addition, local injections of 50 U and 100 U BoNT/A show equal efficacy. Therefore, 50 U of BoNT/A can be used clinically for the treatment of mild to moderate depression. However, this study had some limitations. First, this study had no control arm. Secondly, the study had a small sample size. Therefore, further studies should follow-up patients for a longer period and determine if lower doses show similar efficacy to 50 U of BoNT/A.

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CRediT authorship contribution statement

Haiyang Shu: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft. **Tingting Shen:** Conceptualization, Data curation, Investigation, Writing – original draft. **Wenjing Deng:** Investigation, Visualization. **Jiaqian Cao:** Resources, Supervision. **Yingying Xu:** Software, Validation. **Jing Liu:** Visualization, Writing – review & editing. **Xuping Zhou:** Resources, Supervision, Writing – review & editing. **Wei Feng Luo:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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