

Comparative Study of Bloodletting Therapy and Acupuncture Treatment on Headache Caused by “Blood-Fire Origin”

Bao Lian Sheng¹, Byambasuren Dagvajantsan², Alimaa Tugjamba¹, Tserendagva Dalkh¹

¹Department of Internal Medicine, International School of Mongolian Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia;

²Department of Neurology, School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

Submitted: January 29, 2019

Revised: February 25, 2019

Accepted: March 25, 2019

Corresponding Author

Alimaa Tugjamba, MD, PhD
Department of Mongolian Medicine
Study, International School of
Mongolian Medicine, Mongolian
National University of Medical
Sciences, Ulaanbaatar 13270,
Mongolia

Tel: +976-99171212

E-mail: alimaa.t@mnums.edu.mn

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/bync/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. Copyright© 2019 Mongolian National University of Medical Sciences

Objective: The study aimed to compare the effects of bloodletting and acupuncture treatment on patients with headaches caused by “blood-fire origin”. **Methods:** A cross-sectional study was carried out at Affiliated Hospital of National University of Inner Mongolia, with two treatment groups, one receiving bloodletting and the other acupuncture. The effects of both treatments were evaluated using the traditional medical questionnaire, visual analog scale scores, and serum lab tests at baseline and one month after treatment. Sixty patients with headaches caused by “blood-fire origin” participated in the study and 27 were male while 33 were female. The data were analyzed at baseline and 1 month after treatment using t-tests. **Results:** After treatment, all complaints related to headaches improved in both study groups when compared before the treatment ($p < .05$). The visual analog scale pain score was significantly reduced ($p = .002$) both groups after treatment. Following treatment, the serum nitric oxide and endothelin levels were significantly reduced ($p = .0001$) compared to pre-treatment levels. **Conclusion:** Bloodletting and acupuncture decreased the frequency of headache score and reduced the intensity of pain score in patients with headaches. The nitric oxide and endothelin levels were significantly decreased in patients who received bloodletting treatment compared to acupuncture.

Keywords: Headache, Bloodletting Therapy, Acupuncture Treatment, Questionnaire, Nitric Oxide

Introduction

In Mongolian traditional medicine, headaches caused by “blood-fire origin” are one of the most commonly encountered chronic disorders of nerves and blood vessels. Headache caused by “blood-fire origin” occurs with high prevalence and is typically

poorly treated in traditional medicine. There are many etiologies associated with headache^{1,2}.

In traditional medical source books, headaches are classified into different categories based on the theory of wind, bile and phlegm balance. Bile and blood type headaches are caused by bile and blood-inducing habits (too much hot/spicy/fried foods,

exposure to sunlight/heat, profuse sweating, intensity/anger, etc). These conditions are the main reason for headaches caused by “blood-fire origin”^{1,2}.

The World Health Organization (WHO) pointed out that headaches are a disease of high prevalence among people of any age regardless of their geographical location. It refers to not only a painful sensation in the head but it also often implies dysfunction of the whole body. According to the results of some research, up to 90 percent of the people are affected by headache^{3,4}.

Headaches can occur as a result of many conditions whether severe or not. There are many different classification systems for headaches. The most well-recognized classification is the one published by the International Headache Society⁴. More than 90% of headaches are primary headaches, and they usually occur in patients between 20-40 years old of age. The most common types of primary headaches are migraines and tension headaches. Approximately 64–77% of people have a headache at some point in their lives⁵.

Almost half of the worldwide adult population have one or more types of headaches during their lifetime. Primary headaches are more difficult to understand than secondary headaches. The exact mechanisms which cause migraines, tension headaches and cluster headaches are not well understood. There have been different hypotheses over time which attempt to explain what happens in the brain to cause these headaches⁴⁻⁶.

Recent studies have highlighted a potentially important role for endothelin-1 (ET), a potent vasoconstrictor peptide, in migraine path physiology⁷⁻⁹. Also, nitric oxide (NO) has been proposed to be a key molecule in migraine. A potential role for NO has also been suggested in the pathophysiological mechanisms underlying cluster and chronic tension-type headaches. Further research concerning the role NO in primary headaches are needed to identify reliable markers of NO metabolism and verify NO effects¹⁰.

Chronic headaches are one of the leading issues of public health because they have adverse effects on patients’ ability to work and their quality of life. Patients with headaches tend to use many medications to obtain relief, including some without a prescription, and this can result in drug overdosage⁶.

Recently, headache sufferers have begun paying serious attention to non-pharmaceutical headache management, with several articles concerning on acupuncture traditional

medicine therapies published in many countries. Treatment with the insertion of needles belongs to the group of non-medical methods of treatment and is often used in combination with other techniques of therapy and prophylaxis. Acupuncture is commonly used for the treatment of disorders of the regulating systems of wind, bile, and phlegm. Currently, acupuncture is widely used in medical and prophylactic establishments. The mechanism of its beneficial effect is related to stimulation of biologically active points, connected with the regulating systems for separate organs and tissues¹¹⁻¹³.

Traditional medical source books emphasize that headaches caused by “blood-fire origin” was mainly treated using medication or bloodletting therapy^{2,14}. Bloodletting therapy is an ancient treatment used in Mongolian traditional medicine and has been used not only for headaches but is one of the most widely used traditional medicinal methods in Mongolia for treating hypertension, neck pain, headaches, chronic hepatitis, ophthalmic, and skin conditions and infectious diseases^{1,2,14}.

In the past few years, headaches caused by “blood-fire origin” has been treated using bloodletting therapy with medicine. According to opinions of Mongolian traditional medicine physicians, bloodletting improves blood and is used following strict indications and accompanied with the administration of other drugs. One can suggest that bloodletting used in Mongolian traditional medicine, being different from the Western medical care, results in a change of metabolism in tissues and the results in interrelations tissues and the activated system of homeostasis¹⁴.

However, to our knowledge, no studies have assessed the effectiveness of bloodletting therapy for headaches in comparison to acupuncture. This study aimed to compare the effectiveness of bloodletting therapy and acupuncture treatment in patients with headaches caused by “blood-fire origin” and the impact of these treatments on ET and NO pathophysiology.

Materials and Methods

Study design

A cross-sectional study design was used. Sixty patients diagnosed with headaches caused by “blood-fire origin” were evaluated before and after bloodletting therapy or acupuncture treatments at the Affiliated Hospital of the National University of Inner Mongolia between July 2017 and May 2018. The patients

were divided into 2 groups, the bloodletting group (n=30) and acupuncture (n=30) treatment.

Patients selection

Patients were included in the study who had headaches caused by "blood-fire origin" or primary headaches and were between 18 and 60 years of age. Study exclusion criteria were other types of secondary headaches, psychiatric disorders, cranial trauma or operation, pregnancy, lactating, malignancy, blood coagulation disorders. Patients who had low blood pressure, anemia, or other contraindications were also excluded from bloodletting treatment.

Assessment tools

The primary outcome was assessed using a traditional Mongolian medicine version of the headache disability assessment questionnaire which gathered information about the patient's demographic data, headache characteristics including its frequency, duration, pulsing head pain, nausea, vomiting and photophobia (sensitivity to light) and other symptoms determined to be important by Inner Mongolian Association of Mongolian Medicine¹². The scientific value of this questionnaire was confirmed previously by content value method and the feasible value by the test-retest method. It is a simple and easy to implement method to assess headaches and was modified slightly for our study. We used it to collect data before and after treatments.

Secondary outcomes were pain intensity as measured using a visual analog scale (VAS), and nitric oxide (NO) and endothelin (ET) from blood samples. V.supraorbitalis dextra (right supraorbital vein) and v.supraorbitalis sinistra (left supraorbital vein) were the locations where bloodletting was applied while the acupuncture treatment group received acupoints on the forehead and foot. The acupoints (Bai Hui (GV20), Cuan Zhu (BL2), and Yi Feng (TE17), Tai Yang (Ex-HN5), Shuai Gu (GB8), Yin Tang (Ex-HN3), He Gu (LI4), San Yin Jiao (SP6), Tai Chong (LR3) were selected according to the guidelines of State Standard Name and Location of Acupoints (GB 12346-2006), People's Republic of China¹¹.

Blood sampling

Five milliliters of fasting blood was collected by venous arm puncture under aseptic conditions for lab tests. Serum was obtained by centrifugation at 2,000 g for 5 min of blood samples taken without anticoagulant. Serum was stored at 20 °C until the analysis date.

Determination of NO and ET concentration in serum

NO and ET were extracted using acetone from 1 µl of plasma and subjected to a sensitive enzyme-linked immunosorbent assay. NO and ET concentrations were measured by means of immunoassay (Infinite M200 PRO multimode plate reader; Tecan Trading AG, Austria) with reference to a standard curve. NO levels were expressed as ng/µl while ET levels were expressed in ng/l.

Statistical analysis

All data were analyzed using SPSS 19.0 software and Graph Pad Prism-5 software and descriptive data are presented as the mean±SD or percentage. Pre- and post-treatment differences were calculated by using paired t-tests. Independent t-tests were used to determine the differences between the bloodletting and acupuncture treatment effects. The chi-square test was used to compare the gender frequency distribution of the treatment groups. A value of p<.05 was considered statistically significant for all tests.

Ethical statements

Approval for the study was granted by the Ethical Review Committee of National University of Inner Mongolia (no: NM-LL-2015-07-16-01). After receiving a detailed explanation of the treatment methods, the participants provided written informed consent.

Results

Sixty patients with headaches caused by "blood-fire origin" participated in the study with 27 male patients and 33 female patients. The average age of the patients was 36.78±10.9 years in the bloodletting group and was 37.22±12.29 in the acupuncture group. There were no significant differences in the age, gender, and duration of headaches (p=.148, Table 1).

After treatment, the frequency of headache, duration of headache, and pulsing head pain scores significantly improved in

Table 1. Comparisons of the groups according to the age, gender, and duration of headaches.

Groups	n	Mean age (years)	Gender		Duration of headaches (years)
			F (n)	M (n)	
Bloodletting	30	36.78±10.9	19	11	4.4±5.1
Acupuncture	30	37.22±12.29	14	16	2.8±2.5
p-value		.148 [†]		.19 [†]	.078 [†]

Data were expressed as Mean±SD; Age, gender distribution, and duration of headaches were compared for the treatment groups at the baseline. [†]p-value using independent t-test, *p-value using chi-square test.

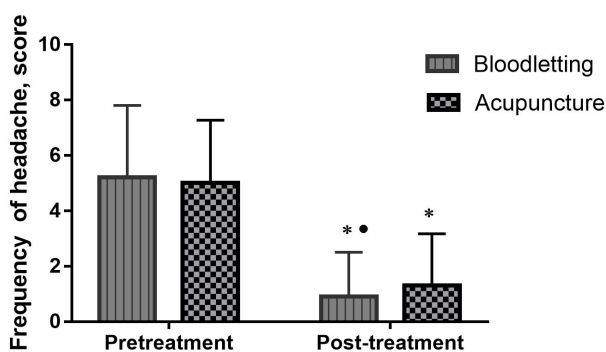


Figure 1. Effects of the bloodletting therapy and acupuncture treatments frequency of headache score.

The frequency of headache scores were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. The significance level was p<.05. Data are presented as mean±SD. *p<.0001 post-treatment vs. pretreatment. *p<.05 bloodletting vs. acupuncture group after treatment.

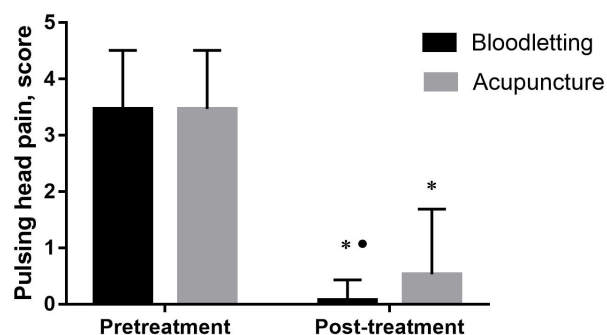


Figure 3. Effect of bloodletting therapy and acupuncture treatments on pulsing head pain score.

The pulsing head pain scores were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. Data are presented as mean±SD. *p<.0001 post-treatment vs. pretreatment. *p=.009 bloodletting vs. acupuncture group after treatment.

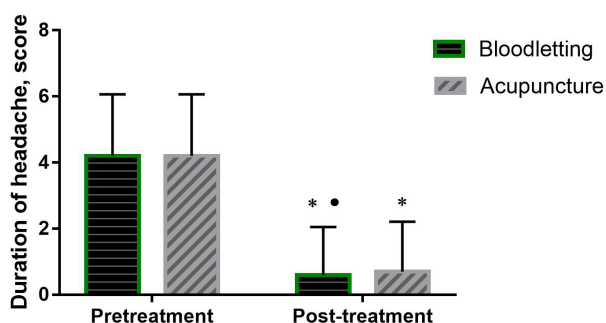


Figure 2. Effects of the bloodletting therapy and acupuncture treatments duration of headache score.

The duration of headache scores were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. Data are presented as mean±SD. *p<.0001 post-treatment vs. pretreatment. *p<.05 bloodletting vs. acupuncture group after treatment.

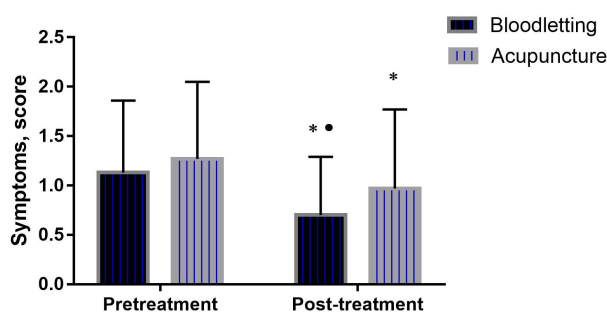


Figure 4. Effects of the bloodletting therapy and acupuncture treatments in symptoms nausea, vomiting, and photophobia (sensitivity to light).

The assessment scores for symptoms of nausea, vomiting and photophobia were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. Data are presented as mean±SD. *p<.0001 vs. pretreatment level. *p<.05 bloodletting vs. acupuncture group at after treatment.

both groups compared before the treatment ($p < .0001$). However, the scores for the frequency of headache in bloodletting group were modestly but significantly decreased compared to the acupuncture group ($p < .05$, Figure 1).

After treatment, the scores for the duration of headache were slightly but significantly decreased in the acupuncture treatment group compared to the bloodletting group ($p < .05$, Figure 2).

Compared to the bloodletting group, the assessment scores for pulsing head pain were significantly decreased in the bloodletting treatment group ($p = .009$, Figure 3).

During follow up, the assessments scores for symptoms (nausea, vomiting, and photophobia) were significantly decreased to pre-treatment level in both group ($p = .0001$, Figure 4)

The effects of the bloodletting therapy and acupuncture treatments were evaluated by using the visual analogue scale before and after treatment. VAS scores for bloodletting and acupuncture were significantly decreased after treatment ($p < .001$) however it was lower in the bloodletting group (2.54 ± 1.23 vs. 4.14 ± 2.01 , $p = .002$, Table 2).

NO levels before and after the treatment in the bloodletting and acupuncture groups were significantly decreased ($p = .043$). During follow up, the NO levels were significantly reduced to

$29,78 \text{ ng}/\mu\text{l}$ (95% CI 29.3-30.21) in the bloodletting group and $33,05 \text{ ng}/\mu\text{l}$ (95% CI. 33.5-34.57) in the acupuncture group compared to pre-treatment levels ($p = .0001$, Table 3).

Plasma levels of ET before and after the treatment in the bloodletting and acupuncture groups were significantly decreased ($p = .0001$). During follow up, the ET level was significantly reduced to 80.45 ng/l (95% CI. 78.32-81.77) in the bloodletting group and 84.69 ng/l (95% CI. 84.78-87.43) in the acupuncture group compared to pre-treatment level ($p = .0001$, Table 3).

Discussion

The results of this trial help clarify the value of bloodletting therapy and acupuncture treatment as treatments for headaches caused by "blood-fire origin" and highlight the differences in the efficacy of these treatments. In the treatment of headaches, in addition to medications, traditional non-Western medical methods are in widespread use.

To study the effects of bloodletting, we chose v.supraorbitalis dextra and v.supraorbitalis sinistra as locations for the bloodletting therapy to treat headaches caused by "blood-fire origin." Therefore, there is a need to conduct research on headaches caused by "blood-fire origin", to develop criteria for

Table 2. Effect of bloodletting and acupuncture on headaches caused by "blood-fire origin".

VAS Score	Bloodletting (n=30)	Acupuncture (n=30)	p-value
Pretreatment	7.83 ± 1.36	7.57 ± 1.1	.409**
Post-treatment	$2.54 \pm 1.2^*$	$4.14 \pm 2.01^*$.002**

The visual analog scale score (VAS) scores used for evaluating the severity of headaches were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. Data expressed as Mean \pm SD; *Independent t-test; **Paired t-test $p < .05$.

Table 3. Comparisons of the effect of bloodletting and acupuncture on headaches on nitric oxide and plasma endothelin levels.

Variables	Bloodletting (n=30)	Acupuncture (n=30)	p-value
NO (ng/ μ l)	Pretreatment	40.31 ± 1.29	.043**
	Post-treatment	$29.78 \pm 1.19^*$.0001**
ET (ng/l)	Pretreatment	106.54 ± 6.72	.0001**
	Post-treatment	$80.45 \pm 4.58^*$.0001**

The nitric oxide (NO) and plasma endothelin (ET) levels were compared at the baseline, after one month after treatment using paired t-tests. Independent t-tests were used to compare the bloodletting and acupuncture treatment effects. Values presented as Mean \pm SD, ** Independent t-test; *Paired t test $p < .05$

diagnosing this disorder, to evaluate outcomes of bloodletting therapy.

We found that pain reduction is significant after bloodletting therapy. This is the same result as the study of Ahmadi et al. which showed that bloodletting leads to clinically relevant benefits for primary care patients with headache¹⁵.

In our study, the effects of both treatments were evaluated using a traditional medical questionnaire before and after treatment regarding the symptoms of pulsing head pain, nausea, vomiting, and photophobia. These symptoms, which occur in headaches caused by blood-fire origin, were decreased significantly in the bloodletting group compared to the acupuncture group. The intensity of the headaches score was also decreased by 4.14 ± 2.01 in the bloodletting group and 2.54 ± 1.23 in the acupuncture group after 30 days which indicates that it may be useful as a treatment for mid to severe intensity migraine headaches. The guidelines for international headache treatments recommended prophylaxis drug treatment for chronic migraine attacks¹⁶. In our study, we could recommend bloodletting treatment for chronic primary headaches and drug-induced headaches based on following result. The headache frequency score decreased by 5 ± 2.27 in bloodletting group and 1.3 ± 1.87 in acupuncture group after 30 days.

The results of this study have shown that bloodletting therapy is more effective at reducing VAS scores than acupuncture treatment. VAS scores were significantly decreased before and after treatment for both treatments ($p = .002$). However, during follow up, the symptoms scores in the bloodletting group were significantly decreased compared to the acupuncture group ($p < .05$).

Uyanga et al. in 2017 reported that acupuncture treatment decreases the frequency of headaches and reduces the intensity of pain and has a positive effect on the quality of life of people who suffer from primary headaches¹⁷. Also, acupuncture treatment has the potential to reduce disability caused by primary headaches and alleviates the depression related to them. That study differed from our research in that we compared acupuncture treatment and bloodletting therapy.

Since drug therapy for migraines headaches has many side effects, other methods that have fewer complications should be used for pain relief when possible. According to some studies, some patients with headaches prefer to be treated by nondrug therapy such as bloodletting, acupuncture, cold therapies,

and etc¹⁸. Bloodletting can be used in the management of headaches. It can be applied as a single form of care, or as part of a comprehensive treatment program.

Also, our results suggest that bloodletting therapy can be effectively applied in the management of headaches. The results of this study have shown that bloodletting therapy is effective at reducing VAS scores and questionnaire pain intensity scores in headaches caused by "blood-fire origin" and that it has a larger effect on questionnaire scores for patients receiving bloodletting therapy and compared to acupuncture.

Iljazi et al. have reported that vasoactive peptides play a key role in the attack-initiating cascade of migraine¹⁹. Elevated endothelin-1 plasma levels have been reported in the early phase of migraine attacks. Our study showed plasma levels of ET after the treatment in the bloodletting and acupuncture groups were significantly decreased ($p = .0001$).

Nitric oxide (NO) is a crucial molecule in headaches by its regulation of cerebral and extracerebral cranial blood flow and arterial diameters^{20,21}. It is also involved in nociceptive processing. Furthermore, the NO-induced delayed headaches have many of the characteristics of primary headaches. This suggests that NO contributes to the mechanisms of several types of primary headaches and that NO-related central sensitization may be an important common denominator in the pain mechanisms of primary headaches²².

It has been proposed that nitric oxide plays a key role in migraine headaches. Experimental evidence suggests its intervention in vasodilatation and activation of the trigeminovascular system as well as its involvement in the supraspinal pathways implicated in pain processing. Other findings imply that NO is involved in coupling neuronal and vascular changes during spreading depression²³.

Our study showed NO levels were significantly decreased after the treatment in both treatments ($p = .0001$). This is particularly important as bloodletting therapy has long been advocated as an effective form of pain relief and that effect is supported by the findings in this study. A purpose of this trial was to determine whether bloodletting therapy was more effective than acupuncture treatment in relieving headaches caused by "blood-fire origin". Although our results showed that bloodletting therapy is effective in headaches treatment, its mechanism of the action is unknown. The reduction in severity and frequency of headaches may be due to decreasing of NO and

plasma ET levels. This study, therefore, contributes to providing a solid foundation for clinical treatment of headaches caused by "blood-fire origin", as well as future research in bloodletting therapy.

Despite the valuable results, our study had some limitations. First, we do not know long term effects of bloodletting therapy and acupuncture treatment as there was only short term follow up in our study. Second, the number of participants in our study was only 60, limiting the conclusions we could draw. Third, the levels of some other pain mediators were not evaluated additionally. Also, the VAS score is a subjective evaluation and error may occur result of variation in headache intensity among the participants. In the future, we should use bloodletting therapy in the treatment of headaches with other alternative treatments by adding it as a treatment in the headache treatment guidelines. The results of the current study indicate that adding bloodletting therapy to the treatment of headaches reduces the pain and number of attacks and can be considered a treatment option for patients willing to undergo this treatment.

Conclusion

Bloodletting and acupuncture treatment decrease the frequency of headaches and alleviate the intensity of pain in patients with headaches caused by "blood-fire origin". The NO and plasma ET level were significantly decreased among patients who received bloodletting treatment compared to acupuncture, suggesting the possible mechanism of bloodletting treatment effects.

Conflict of Interest

The authors state no conflict of interest

Acknowledgement

The authors provided no information regarding financial or institutional support or who contributed to their study.

References

1. Tumbaa B. Four roots of medicine. Ulaanbaatar, Mongolia: State Printing; 1991. p 53-55.
2. Tumurbaatar N, Tserendagva D, Lagshmaa B, Oldokh S. Method of diagnose and treatment of headache in Eastern and Western medicine. Ulaanbaatar, Mongolia: Erkhes printing; 2001. p 89-90.
3. Tsagaankhy G. Neurology. Ulaanbaatar, Mongolia: Admon press; 2011. p 302-312.
4. Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia 2013; 33: 629-808. doi: 10.1177/0333102413485658.
5. Stovner LJ, Andree C. Prevalence of headache in Europe: a review for the Euro light project. J Headache Pain 2010; 11: 289–99.
6. Rossi P, Di L, Faroni J, Cesarino F, Nappi G, Di Lorenzo C. Advice alone vs. structured detoxification programmers for medication overuse headache: a prospective, randomized, open-label trial in transformed migraine patients with low medical needs. Cephalalgia 2006; 26: 1097–105.
7. Iljazi A, Ayata C, Ashina M, Hougaard A. The Role of Endothelin in the Pathophysiology of Migraine-a Systematic Review. Curr Pain Headache Rep 2018; 22: 27.
8. Heshmat-Ghahdarjani K, Javanmard SHH, Sonbolestan SA, Saadatnia M, Sonbolestan SA. Endothelial Function in Patients with Migraine without Aura during the Interictal Period. Int J Prev Med 2015; 6: 2. doi:10.4103/2008-7802.151432.
9. Franceschini R, Tenconi GL. Endothelin-1 plasma levels in cluster headache. Headache 2002; 42: 120-4.
10. Gallai V, Sarchielli P. Nitric oxide in primary headaches. Headache Pain 2000; 1: 145-54.
11. Xuemin SH. Chinese Acupuncture and Moxibustion. Shanghai, China: Shanghai University of Chinese Medicine Press; 2002. p 123-128.
12. ZhangAn li, Yang ZC. English Chinese prescriptions of China acupuncture and moxibustion 4th edition. Shanghai, China: Shanghai University of TCM press; 2014. p 125-127.
13. Endres HG, Bowling G, Diener HC, Lange S, Maier CH, Molsberger A, et al. Acupuncture for tension-type headache: a multicentre, sham-controlled, patient-and observer-blinded, randomised trial. J Headache Pain 2007; 8: 306-14.
14. Tserendagva D. Study of effect of bloodletting therapy

- [dissertation]. Ulaanbaatar, Mongolia: National Medical University of Mongolia; 2000.
15. Ahmadi A, Schwebel DC, Rezaei M. The efficacy of wet-cupping in the treatment of tension and migraine headache. *Am J Chin Med* 2008; 36: 37-44.
 16. Tassorelli CH. Guidelines of the International Headache Society for controlled trials of preventive treatment of chronic migraine in adults. *Neuropsychiatr Dis Treat* 2019; 9: 709-20. doi: 10.2147/NDT.S33769.
 17. Uyanga E. Results of acupuncture treatment on primary headache [dissertation]. Ulaanbaatar, Mongolia: Mongolian National University of Medical Sciences; 2017.
 18. Shahrakivahed A, Firouzkouhi M, Moushtagh Z. The effect of bloodletting on migraine headache. *JIARM* 2014; 8: 21-6.
 19. Iljazi A, Ayata C, Ashina M, Hougaard A. The Role of Endothelin in the Pathophysiology of Migraine—a Systematic Review. *Curr Pain Headache Rep* 2018; 22-7.
 20. Yong-Lie Z. Role in nitric oxide in the pathogenesis of migraine. *Zhongguo linchaung kangfu* 2006; 7: 138-9.
 21. Hongya Z. Changes of nitric oxide and vascular endothelin levels in peripheral blood of patients with migraine. *Zhongguo linchaung kangfu* 2007; 10: 121-2.
 22. Olesen J. The role of nitric oxide (NO) in migraine, tension-type headache and cluster headache. *Pharmacol Ther* 2008; 120: 157-71. doi: 10.1016/j.pharmthera.2008.08.003.
 23. Ashina M, Bendtsen L, Jensen R, Olesen J. Nitric oxide-induced headache in patients with chronic tension-type headache. *Brain* 2000; 123: 1830-7.