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Original Research

The Effectiveness of Leech Therapy in the Severity of Diabetic Neuropathy: A Randomized Controlled Trial

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Abstract

Diabetic peripheral neuropathy is one of the most common causes of disability in diabetic patients. Applying a method to achieve the highest therapeutic effect in patients is desirable. Therefore, this study aimed to evaluate the impact of leech therapy on diabetic neuropathy of lower limbs in comparison to gabapentin as a standard method. This randomized controlled parallel-group clinical trial was conducted among 40 patients with type II diabetes who were diagnosed with lower limb diabetic neuropathy and referred to specialized outpatient clinics in Babol, Mazandaran province, Iran from 23 September 2020 to 17 March 2021. The patients were randomly divided into two groups. One group was treated with leech therapy and the other group was treated with gabapentin as the standard method. The severity of neuropathy was assessed every 15 days until the 45th day. The Visual Analog Scale (VAS), Neuropathy Symptom Score (NSS), Neuropathy Disability Score (NDS), and Nerve Conduction Velocity (NCV), and Electromyography (EMG) were used for assessing the study outcomes. The repeated measure and Friedman tests were used by SPSS.V.23. The results of our study indicated that pain (P value:0.03), numbness (P value<0.0001), and paranesthesia (P value:0.01) significantly reduced in patients undergoing leech therapy versus patients taking gabapentin on the 45th day. The total NSS (P value<0.0001) and total NDS (P value<0.0001) improved significantly for patients with leech therapy over 45 days compared to the patients with gabapentin. The results of our study showed that using leech therapy for patients with diabetic neuropathy was more effective in improving clinical symptoms and the functions of lower limb muscles and nerves in comparison to gabapentin. The severity and symptoms of neuropathy greatly improved for the patients treated with leech therapy versus patients taking gabapentin.

Keywords: Diabetes; Gabapentin; Iran; Leech therapy; Neuropathy; Persian medicine

Introduction

Diabetic peripheral neuropathy is one of the most common causes of disability in diabetic patients and is a progressive and irreversible symptom. Following nerve damage due to vascular disorders, the failure and reduction of axons due to various mechanisms may cause tissue damage [1]. Peripheral nerves disorders occur in 25% of people with diabetes within 10 years of diagnosis. The decreased sensation associated with diabetic peripheral neuropathy is implicated in the disturbance of balance, walking pattern, and increased risk of falls [2].

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In diabetic patients, these disorders affect the quality of life and increase with age over 60, high blood pressure, hyperlipidemia, smoking, ischemic heart disease, and poor control of diabetes [3]. Peripheral neuropathy is the first stage of diabetic foot ulcer which, if not detected immediately, may progress to amputation and therefore the risk of amputation in diabetic peripheral neuropathy increases by 15% [3].

Pain is a common symptom in more than 30% of patients with diabetic neuropathy [4]. Symptoms are usually found in the lower extremities, initially on the soles of the feet and toes, and cause discomfort and affect all aspects of people's lives, including their mood, sleep, mobility, ability to work, and social relationships [1].

For the treatment of diabetic peripheral neuropathy, symptomatic therapies are mainly used, with a success rate of less than 40 to 60%. Thus, non-pharmacological treatments have been suggested for this disorder [1,5]. The most effective way to stop this process is to increase blood circulation in damaged tissues without the risk of blood clots [6]. Various studies have suggested that leech therapy can play a pivotal role in wound healing as leeches have healing properties in the saliva, containing an anticoagulant called hirudin, which opens clogged arteries and increases the blood flow [7-9]. Some studies recommend that leech therapy can be safe and effective in controlling chronic migraines and headaches [10]. Moreover, in the traditional Persian medicine, leech therapy is recommended to improve some skin diseases and local blood circulation in the limbs and organs, and currently, leeches are used for the treatment of vascular disorders, especially healing wounds and skin cramps [11,12].

Since neuropathy is one of the most common complications in diabetic patients causing numbness and impaired perception of deep sensation, especially in lower extremities, it exposes patients to many problems. Several studies have addressed the effect of leech therapy on diabetic neuropathy [13,14]. Thus, it is necessary to use a combination of medications and methods to achieve the highest therapeutic effect in patients. Therefore, this study aimed to evaluate the effectiveness of leech therapy in diabetic neuropathy of lower limbs in comparison to gabapentin as a standard method.

Materials and Methods

Subject and setting

This randomized controlled parallel-group clinical trial (registration No: IRCT20150927024228N3) was conducted among patients with type II diabetes who were diagnosed with lower limb diabetic neuropathy and referred to specialized outpatient clinics in Babol, Mazandaran province, Iran from 23 September 2020 to 17 March 2021. In this study, patients with HbA1c ≥ 6.5% (≥ 48 mmol/mol) or random plasma glucose ≥ 200 mg/dL (≥ 11.1 mmol/L) or fasting plasma glucose ≥ 126 mg/dL (≥ 7.0 mmol/dL) or OGTT 2 hour glucose in venous plasma ≥ 200 mg/dL (≥ 11.1 mmol/L) were considered as type II diabetes [15,16]. Diabetic neuropathy was defined as peripheral nerve damage or dysfunction [17]. The scores of the two questionnaires, Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS), determined peripheral diabetic neuropathy. Obtaining at least 6 scores of NDS regardless of NSS score or at least 5 scores of NSS in combination with 3-5 scores of NDS was considered as the criteria for peripheral diabetic neuropathy [18].

The inclusion criteria encompassed A) Age of more than 30 years, B) Lack of pregnancy and lactation, C) Lack of other diseases and situations that caused neuropathy such as alcohol abuse, vitamin B12 deficiency, chemotherapy, kidney failure, nerve compression, autoimmune diseases, infection and Guillain-Barre syndrome, and D) Insensitivity to gabapentin and leech therapy. We excluded patients with diabetic foot ulcers who required surgery and suffered from anemia and blood coagulation diseases. In addition, patients who used anticoagulants such as heparin and warfarin and were hospitalized due to cardiovascular diseases were excluded from the study. In a similar vein, patients who used painkillers and other routine medications for neuropathy one month before the initiation of the study were not included. The side effects of leech therapy were itching, swelling, spontaneous bleeding, and infection at the leech therapy site. Patients with side effects treated with antibiotics and conservative treatment were also excluded from the study.

Sample size and sampling

The sample size for this study was estimated using repeated measure, within-between interaction by G*power software. The effect size was considered as 30% based on a related study [9]. By taking into account the 80% study power, a 95% confidence interval (CI), 5% error level, and the number of repetitions (4 times), the sample size was calculated to be 18 patients. Considering that 10% of patients might drop out during the follow-up period, 20 diabetic patients were considered as the sample size in each group.

Randomization and blinding

The patients were randomly divided into two groups by random number sequence based on patient order entry. The randomization was concealed by sequentially numbered, opaque sealed envelopes (SNOSE). In the present study, the nurse who allocated patients and referred them to the physician's room was blind-

ed to the procedure. The physician who assessed the severity of peripheral diabetic neuropathy in physical examination sessions was also blinded. The data analyst did not perform the allocation of patients and was also blind. Conversely, the patients were not blind to the procedure.

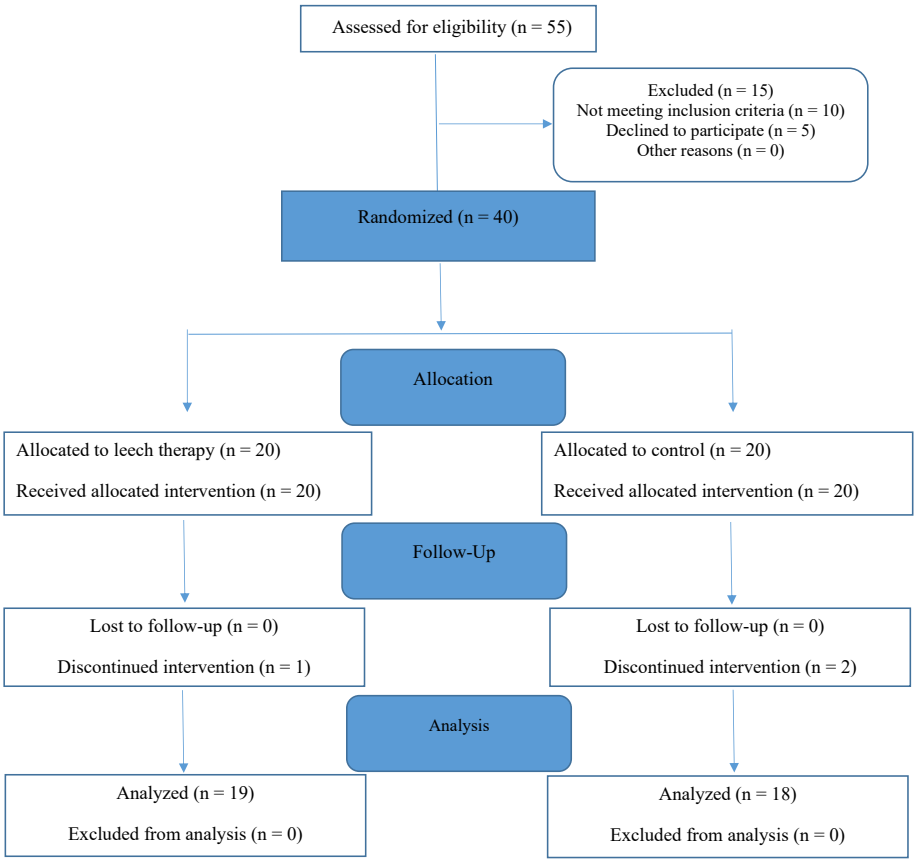

Figure 1. The flow diagram of the study

Intervention

The feet of the patients in the intervention group (leech therapy) were washed with warm water and dried. Then, 3-5 median natural leeches (leech size: 6-8 cm) were located on the back of both feet (tangentially from the root of the second toe to the external malleolus). The leech therapy was performed in 3 phases at 15-day intervals. The patients in the control group received a 300-mg capsule of gabapentin per day before sleeping for 30 days.

Data collection

The data were collected through physical and clinical examinations. We followed up the patients for 45 days. At baseline (the first day of the study before intervention), the severity of neuropathy was assessed by 4 methods.

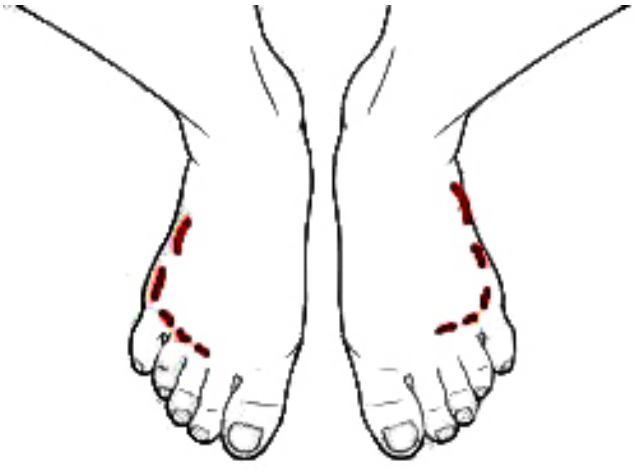

Figure 2. The positions of leeches on the back of legs

1. Visual Analogue Scale (VAS): The severity of pain, burning, numbness, and paranesthesia was assessed by using VAS. This graded scale measures the severity of events in the range of zero to ten scores. The higher scores indicate greater severity of the measured outcomes.
2. Neuropathy Disability Score (NDS): In this scale, 1) the vibration at the top of the big toe was examined by diapason 128 HZ. 2) Feeling of heat and cold at the back of the feet was checked by an ice bag and test tube containing hot water. 3) Sharp or blunt instrument at top of the big toe. 4) Presence or absence of Achilles tendon reflexes (present: 0; present with reinforcement: 1, and absence: 2). Any items checked for both feet abnormality had 2 points and normality had 0 point. The range of scores for this questionnaire was 0 to 10, with higher scores showing the severity of peripheral diabetic neuropathy [19].
3. Neuropathy Symptom Score (NSS): This questionnaire covers four aspects of peripheral diabetic symptoms and its range of score is from 0 to 10. The first part of the questionnaire assesses the symptomatology of diabetic neuropathy. In this part, burning/numbness/paranesthesia had 2 points, and pain had 1 point. In addition, fatigue/cramps had 1 point in the lower limbs. The maximum score for this section was 3. The second part of the questionnaire addresses the localization of symptoms. Presentation of symptoms in feet had 2 points or 1 point in the lower leg. In the third part of the questionnaire, the exacerbation of symptoms during the night had 2 points, or its presence during the day and night had an equally 1 point. If symptoms awake patients from sleep, 1 point is added to the third part. If symptoms improve due to walking and standing, 2 and 1 points are recorded in the last part of the questionnaire [20].
4. Electromyography and Nerve Conduction Velocity (EMG-NCV): The function of motor and sensory nerves as well as muscles in the lower limbs were assessed by Nerve Conduction Velocity (NCV) and Electromyography (EMG), respectively. The activities of medial gastrocnemius, tibialis anterior, and vastus lateralis muscles were measured by EMG. For checking and recording the EMG signals, bipolar electrodes were amplified by a multichannel differential amplifier with a frequency band ranging from 10 to 10 kHz. The findings of EMG were categorized as normal, isolated spontaneous activity, chronic neurogenic, and acute denervation. In NCV, both the tibial nerve, deep peroneal nerve (DPN), and sural nerve were considered as motor and sensory nerves, respectively. For both the tibial nerve and DPN, the latency and amplitude of nerves were examined. The amplitude was measured from the baseline to the negative peak. The onset latency is the time from the stimulus to the initial negative deflection of the baseline for a biphasic sensory nerve action potential (SNAP) or the initial positive peak for a triphasic SNAP. For each recording, the amplitude was measured from the baseline to the negative peak. Surface electrodes were used for the study. The recording electrodes were fixed to the patient's skin using adhesive tape and the skin was prepared by scrubbing with disinfectant. EMG-NCV was performed by a specialist physician with one machine in a room with a stable temperature during one season.

Primary and secondary outcomes
The decreased severity of pain, burning, numbness, paranesthesia, and decreased scores in NDS and VAS were considered as primary outcomes. Secondary outcomes included decreased scores of NSS, a decrease in latency and an increase in the amplitude of motor and sensory nerves in NCV as well as the function of muscles in lower limbs in EMG. The outcomes related to VAS, NSS, and NDS were checked at baseline and every 15 days. The EMG-NCV was performed only at baseline and 45 days after the intervention.

Ethical approval
The protocol of the present study was reviewed and approved by the Ethics Committee of Kerman University of Medical Sciences (Ethics No: IR.KMU.REC.1399.317). The study was initiated after obtaining informed consent from the patients.

Data analysis
Data were described using mean ± Standard Deviation (SD), frequency, and percentage. The normal distribution of the quantitative variables such as age, duration of disease (diabetes), the VAS, NDS, and NSS scores, and latency and amplitude of NCV were checked by the Kolmogorov-Smirnov test. We used t-test and Mann Withy U test to compare the variables with normal and abnormal distribution between the intervention and control groups. The changes in the VAS, NSS, and NDS scores during different time intervals for each group were checked using Friedman's test. The changes in NCV items before and after the intervention were assessed by the Wilcoxon test. The data analysis was performed by using SPSS software (version 23). The significance level for two-tailed tests was ≤ 0.05.

Results
In the present study, 40 diabetic patients with neuropathy in the lower limbs participated. The mean age of the patients was 54.65 ± 7.62 years (age range: 37 to 72 years). A third-quarter of the patients were female (n=30, 75%) and the mean years of diabetes duration was 15.85 ± 8.55 years (range of duration: 3 to 40). The results showed that the demographic data of the

patients were not significantly different between the intervention and control groups, but some clinical data such as burning score (P value < 0.0001), paranesthesia score (P value < 0.0001), and NDS score (P value=0.04) were significantly different at baseline. The patients in the leech therapy group had higher scores for the clinical variables in comparison to the gabapentin group at baseline (Table 1).

Changes in VAS over different time intervals

According to the results, pain, burning, numbness, and paraesthesia scores decreased significantly for all patients in both groups after 45 days (P value < 0.0001) (Table 2). The pain score was similar for the two groups of patients at baseline (P value=0.47), but this score decreased to a greater extent for the patients undergoing leech therapy versus the patients taking gabapentin at 45 days after the intervention (P value=0.03) (Table 2). The burning score for the patients in the leech therapy group was higher than that of the patients who took gabapentin at baseline (P value<0.0001), but after days, this score improved significantly for the patients in the leech therapy group as was the case for the participants in the gabapentin group in the next time intervals (Table 2).

Table 1. A comparison of baseline characteristics of diabetic patients with neuropathy in lower limbs who were referred to the outpatient clinics of Babol, 2021

Variables	Categories	Leech therapy (n=20)	Gabapentin (n=20)	P value
Age (mean ± SD[1])		55.25 ± 9.61	54.05 ± 5.09	0.62
Duration of disease (mean ± SD)		15.15 ± 8.98	16.55 ± 8.26	0.47
Sex (%)	Male	6 (30)	4 (20)	0.46
	Female	14 (70)	16 (80)	
VAS[2] (mean ± SD)	Pain	5.00 ± 4.05	4.25 ± 3.66	0.47
	Burning	7.90 ± 2.95	4.40 ± 3.26	<0.0001
	Numbness	7.00 ± 3.49	6.50 ± 3.01	0.26
	Paranesthesia	9.05 ± 1.31	5.40 ± 2.50	<0.0001
NDS[3] (mean ± SD)		8.55 ± 1.43	7.35 ± 2.05	0.04
NSS[4] (mean ± SD)		8.70 ± 1.17	8.60 ± 1.42	0.96

1. Standard Deviation (SD); 2. Visual Analogue Scale (VAS); 3. Neuropathy Disability Score (NDS); 4. Neuropathy Symptom Score (NSS)

Table 2. A comparison of VAS scores between diabetic patients in the two groups over different time intervals

Variables	Groups	Time intervals				P value	Mean differences*
		Baseline	15 days later	30 days later	45 days later		
Pain	Leech Therapy (n=19)	5.00 ± 4.05	3.05 ± 3.06	1.73 ± 2.44	0.84 ± 1.74	<0.0001	4.05 ± 3.47
	Gabapentin (n=18)	4.25 ± 3.66	3.40 ± 3.03	3.21 ± 3.11	3.00 ± 3.01	<0.0001	1.05 ± 1.47
P value		0.47	0.71	0.14	0.03	--	0.02
Burning	Leech Therapy (n=19)	7.90 ± 2.95	4.15 ± 2.73	2.42 ± 2.54	1.36 ± 2.19	<0.0001	6.52 ± 3.22
	Gabapentin (n=18)	4.40 ± 3.26	3.65 ± 2.85	2.89 ± 2.62	2.94 ± 2.77	<0.0001	1.33 ± 1.37
P value		≤0.001	0.56	0.62	0.06	--	<0.0001
Numbness	Leech Therapy (n=19)	7.00 ± 3.49	4.80 ± 2.96	2.36 ± 2.38	1.78 ± 2.17	<0.0001	5.26 ± 3.21
	Gabapentin (n=18)	6.50 ± 3.01	5.70 ± 2.84	5.26 ± 2.88	5.33 ± 3.00	<0.0001	1.16 ± 1.24
P value		0.26	0.30	0.02	0.001	--	0.001
Paranesthesia	Leech Therapy (n=19)	9.01 ± 1.31	5.25 ± 2.71	2.94 ± 2.36	1.94 ± 2.17	<0.0001	7.05 ± 2.01
	Gabapentin (n=18)	5.40 ± 2.50	4.50 ± 2.39	3.89 ± 2.30	3.88 ± 2.39	<0.0001	1.44 ± 1.14
P value		≤0.001	0.27	0.23	0.01	--	<0.0001

*Mean difference between baseline and 45days after intervention

The numbness score showed no intragroup and intergroup differences (P value=0.26), but this score decreased significantly for the patients in the leech therapy group compared to the patients in the gabapentin group at the third and fourth-time intervals (P value= 0.02 vs. < 0.0001) (Table 2). The paranesthesia score like the burning score was higher for the patients in the leech therapy group versus the patients in the gabapentin group at baseline (P value < 0.0001), but after 45 days, this score significantly improved for the patients undergoing leech therapy, and these patients obtained a lower paranesthesia score in comparison to the patients in the gabapentin group (P value=0.01) (Table 2).

Changes in NDS over different time intervals

The scores related to the vibration perception threshold for the patients receiving leech therapy were higher than those of the patients in the gabapentin group at baseline (P value=0.03). Over time, this score improved significantly for the patients in the leech therapy group (P value < 0.0001) and these patients had lower scores of the vibration perception threshold versus the patients in the gabapentin group after 45 days of intervention (P value=0.004) (Table 3). The scores related to the perceived dorsum temperature also statistically decreased for the patients in the leech therapy and gabapentin group over different time intervals (P value < 0.0001 vs. P value=0.04), but the patients in the leech therapy group obtained lower scores compared to the patients receiving gabapentin at 30 and 45 days after the intervention (P value < 0.0001 vs. P value=0.003) (Table 3).

Table 3. A comparison of NDS scores between diabetic patients in the two groups over different time intervals

Variables	Groups	Time intervals				P value	Mean difference*
		Baseline	15 days later	30 days later	45 days later		
Vibration perception threshold	Leech Therapy (n=19)	1.85 ± 0.48	0.95 ± 0.94	0.63 ± 0.83	0.21 ± 0.53	<0.0001	1.63 ± 0.68
	Gabapentin (n=18)	1.40 ± 0.82	1.35 ± 0.81	1.10 ± 0.87	1.00 ± 0.90	0.06	0.33 ± 0.76
P value		0.03	0.17	0.09	0.004	--	<0.0001
Temperature perception of the dorsum	Leech Therapy (n=19)	0.95 ± 0.94	0.30 ± 0.57	0	0	<0.0001	1.00 ± 0.94
	Gabapentin (n=18)	0.85 ± 0.81	0.75 ± 0.85	0.68 ± 0.82	0.55 ± 0.78	0.04	0.33 ± 0.68
P value		0.76	0.07	<0.0001	0.003	--	0.02
Pin-prick	Leech Therapy (n=19)	2.00 ± 0.00	1.25 ± 0.91	0.42 ± 0.69	0.21 ± 0.53	<0.0001	1.78 ± 0.53
	Gabapentin (n=18)	1.65 ± 0.67	1.55 ± 0.75	1.57 ± 0.76	1.61 ± 0.77	0.73	0.05 ± 0.53
P value		0.01	0.27	<0.0001	<0.0001	--	<0.0001
Achilles reflex	Leech Therapy (n=19)	3.75 ± 0.71	3.65 ± 0.67	3.42 ± 1.07	3.42 ± 1.07	0.005	0.36 ± 0.59
	Gabapentin (n=18)	3.45 ± 0.82	3.45 ± 0.82	3.42 ± 0.83	3.38 ± 0.84	1	0
P value		0.14	0.44	0.75	0.66	--	0.01
Total NDS	Leech Therapy (n=19)	8.55 ± 1.43	6.15 ± 2.27	4.42 ± 1.73	3.78 ± 1.27	<0.0001	4.84 ± 1.34
	Gabapentin (n=18)	7.35 ± 2.05	7.10 ± 2.17	6.78 ± 1.98	6.55 ± 2.03	0.005	0.72 ± 1.17
P value		0.04	0.18	<0.0001	<0.0001	--	<0.0001

*Mean difference between baseline and 45 days after intervention

The pinprick score improved for the patients with leech therapy over different time intervals (P value < 0.0001), while this score was approximately stable for the patients with gabapentin over different time intervals (P value=0.89). The results also showed that the patients in the leech therapy group had higher pinprick sensation scores at baseline versus the patients in the gabapentin group (P value=0.01), but over different time intervals these scores improved and the patients in the leech therapy group had better pinprick

sensation compared to the patients in the gabapentin group after 30 and 45 days of intervention (P value < 0.0001) (Table 3). Achilles reflex scores was the only variable that was stable for all patients in both groups over different time intervals. According to the results, the NDS scores significantly decreased for all patients over different time intervals, but this decrease showed a significant trend for the patients undergoing leech therapy after 30 and 45 days of the intervention (P value < 0.0001) (Table 3).

Changes in NSS over different time intervals

According to our findings, the severity of neuropathy symptoms such as burning sensation, numbness, cramps, and pain improved for the leech therapy group over different time intervals, while these symptoms were stable for the patients in the gabapentin group (P value < 0.0001 vs. 0.35). The patients undergoing leech therapy had lower symptomatology scores compared to the patients taking gabapentin after 45 days (P value=0.002) (Table 4). The localization score for the patients receiving gabapentin was stable over different time intervals, while this score decreased for the patients undergoing leech therapy (P value=0.004). These patients also obtained better scores than the patients with gabapentin after 30 and 45 days of intervention (P value=0.03 vs. 0.02) (Table 4). The exacerbation of symptoms during night or day was stable for the patients taking gabapentin over different time intervals, while this score statistically decreased for the patients who received leech therapy over different time intervals (P value < 0.0001). There was no significant difference between the two groups in terms of the exacerbation scores at different times (Table 4).

Table 4. A comparison of the NSS scores between diabetic patients in the two groups over different time intervals

Variables	Groups	Time intervals				P value	Mean difference*
		Baseline	15 days later	30 days later	45 days later		
Symptomatology	Leech Therapy (n=19)	2.60 ± 0.50	2.45 ± 0.60	2.21 ± 0.91	1.52 ± 1.17	<0.0001	1.10 ± 1.32
	Gabapentin (n=18)	2.65 ± 0.48	2.65 ± 0.48	2.63 ± 0.49	2.61 ± 0.50	0.35	0
P value		0.74	0.29	0.13	0.002	--	0.001
Localization	Leech Therapy (n=19)	2.00 ± 0.00	2.00 ± 0.00	1.78 ± 0.41	1.73 ± 0.45	0.004	0.26 ± 0.45
	Gabapentin (n=18)	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	NA	0
P value		1	1	0.03	0.02	--	0.02
Exacerbation	Leech Therapy (n=19)	2.25 ± 1.01	2.00 ± 0.97	1.78 ± 1.03	1.42 ± 1.01	<0.0001	0.31 ± 0.74
	Gabapentin (n=18)	2.15 ± 0.93	2.05 ± 0.94	1.89 ± 0.99	1.88 ± 0.90	0.22	0.16 ± 0.51
P value		0.92	0.92	0.91	0.36	--	0.62
Symptom improvement	Leech Therapy (n=19)	1.55 ± 0.68	1.45 ± 0.75	1.26 ± 0.93	1.26 ± 0.93	0.01	0.31 ± 0.67
	Gabapentin (n=18)	1.35 ± 0.93	1.30 ± 0.97	1.26 ± 0.99	1.33 ± 0.97	0.39	0.05 ± 0.23
P value		0.65	0.81	0.93	0.84	--	0.15
Total NSS	Leech Therapy (n=19)	8.70 ± 1.17	8.20 ± 1.32	7.10 ± 1.76	5.78 ± 1.87	<0.0001	2.94 ± 1.89
	Gabapentin (n=18)	8.60 ± 1.42	8.35 ± 1.53	8.15 ± 1.50	8.11 ± 1.23	0.005	0.61 ± 0.91
P value		0.96	0.61	0.06	0.001	--	<0.0001

*Mean difference between baseline and 45 days after intervention

The data revealed that the symptoms improved for the patients in the leech therapy group when they walked, stood, or sat over different time intervals (P value=0.01), but no marked changes was observed in the patients taking gabapentin in these times (P value=0.39). Finally, total NSS improved over different time intervals for all patients, and after 45 days of intervention the patients receiving leech therapy had

a better condition in comparison to the patients who took gabapentin (P value < 0.0001) (Table 4).

Changes in EMG and NCV after the intervention

There were no significant changes in the activities of three leg muscles in the lower limbs based on the EMG findings at baseline between the two groups (P value=0.72) (Table 5), but 45 days after the intervention, the frequency of patients with acute denervation in the gabapentin group was considerably greater than the number of patients with the same complication in the leech therapy group (92.3% vs. 7.7%, P value < 0.0001) (Table 5). The function of the sural nerve as a sensory nerve was stable based on the NCV findings during the study and it was zero (no voltage) for all patients in both groups before and after the intervention. But the function of the tibial nerve and DPN as motor nerves changed for all patients in both groups after the intervention (Table 6). The results of the present study showed that baseline nerve conduction was similar between the two groups, but after the intervention, the amplitude of the tibial nerve in the left foot increased effectively for the patients in the leech therapy group versus the patients in the gabapentin group (P value=0.02). The latency of the tibial nerve in the right and left feet decreased in patients in both groups after the intervention, but the amplitude of the tibial nerve in the right and left feet only increased among the patients receiving leech therapy (Table 5). The latency and amplitude of the DPN among the patients undergoing leech therapy significantly changed. The latency of DPN in the right and left feet decreased significantly in the two groups after the intervention (P value=0.01 vs. 0.002, respectively). The amplitude of DPN in the left foot increased for the patients in the leech therapy group after the intervention (P value=0.02), and the amplitude of DPN in the right foot only increased for the patients in the gabapentin group over time (P value=0.03) (Table 6).

Table 5. A comparison of EMG findings in the patients in the two groups before and after the intervention

Groups	EMG findings (before)				P value
	Normal	Isolated spontaneous activity	Chronic neurogenic	Acute denervation	
Leech therapy	0	0	15 (51.7)	5 (45.5)	0.72
Gabapentin	0	0	14 (48.3)	6 (54.5)	
Groups	EMG findings (after)				P value
	Normal	Isolated spontaneous activity	Chronic neurogenic	Acute denervation	
Leech therapy	0	0	18 (75)	1 (7.7)	0.001
Gabapentin	0	0	6 (25)	12 (92.3)	

Table 6. A comparison of NCV findings in the patients with leech therapy and gabapentin before and after the intervention

Variables	Groups	Pre-intervention	Post-intervention	P value
Tibial latency (Left)	Leech Therapy	5.20 ± 2.46	4.43 ± 2.05	0.02
	Gabapentin	4.61 ± 2.96	3.50 ± 2.79	0.006
P value		0.60	0.34	
Tibial latency (Right)	Leech Therapy	5.32 ± 2.89	3.98 ± 2.19	0.004
	Gabapentin	4.14 ± 2.89	3.49 ± 2.73	0.008
P value		0.11	0.92	
Tibial amplitude (Left)	Leech Therapy	1.88 ± 1.82	3.06 ± 1.93	0.02
	Gabapentin	1.58 ± 1.62	1.70 ± 1.74	0.30
P value		0.45	0.02	
Tibial amplitude (Right)	Leech Therapy	1.61 ± 1.61	2.44 ± 1.90	0.001
	Gabapentin	1.31 ± 1.22	1.64 ± 1.59	0.22

P value		0.82	0.17	
DPN latency (Left)	Leech Therapy	3.27 ± 2.81	2.17 ± 2.27	0.002
	Gabapentin	2.80 ± 3.14	2.80 ± 3.05	0.11
P value		0.56	0.41	
DPN latency (Right)	Leech Therapy	3.45 ± 2.97	2.21 ± 2.34	0.01
	Gabapentin	3.34 ± 3.21	3.11 ± 3.02	0.11
P value		0.91	0.14	
DPN amplitude (Left)	Leech Therapy	0.70 ± 0.94	1.85 ± 2.17	0.02
	Gabapentin	0.85 ± 1.01	0.88 ± 1.03	0.68
P value		0.88	0.33	
DPN amplitude (Right)	Leech Therapy	0.76 ± 0.93	1.47 ± 1.75	0.05
	Gabapentin	0.74 ± 0.95	1.13 ± 1.30	0.03
P value		0.80	0.67	

Discussion

Polyneuropathy is one of the frequent complications among diabetic patients. Polyneuropathy can lead to poor quality of life for patients and there is not a specific and standard method or medication for reducing it. In the present study, we assessed the effect of leech therapy, as a traditional medicine for reducing the severity of polyneuropathy symptoms and also for assessing the function of muscles and motor and sensory nerves in lower limbs. The results of this study indicated that pain, numbness, and paranesthesia were significantly reduced for the patients who received leech therapy versus the patients who took gabapentin over different time intervals. The total NSS and total NDS and some subscales of them improved significantly for the patients in the leech therapy group over different time intervals compared to the patients who were treated with gabapentin. The function of the tibial nerve and DPN changed positively for the patients receiving leech therapy.

There is hardly any existing literature proving the role of leech therapy for healing or improving the severity and symptoms of polyneuropathy, especially among diabetic patients; while one of the uses of leech in traditional Persian medicine is to improve and treat diabetic neuropathy [21]. Many studies have focused on diabetic foot ulcer healing [11,22,23]. One case report in Iran showed that leech therapy can effectively treat diabetic neuropathy and improve the quality of patients' lives [24]. The findings of the present study highlighted that the severity of pain based on VAS and NSS (symptomology) decreased significantly over time in the patients undergoing leech therapy compared to the patients taking gabapentin after 45 days of intervention. There were no similar studies conducted among diabetic patients to assess the role of leech therapy or other traditional medicines in the treatment of neuropathy, but some studies have shown that patients who experienced leech therapy reported lower pain [25,26]. Kalender et al. showed that a cancer patient who suffered from severe pain in the lumbar region two months after leech therapy was referred to the clinic free of pain [27]. A review study by Koeppen et al. revealed the analgesic effect of leech therapy for many conditions. They emphasized that leech therapy is fast pain relief and is effective and long-lasting [28]. Another study that assessed the effectiveness of leech therapy in osteoarthritis of the knee indicated that patients who were treated with leeches had lower pain over time and also compared to the patients in the control group who were treated with diclofenac as a standard method [29]. The results of the present study were in line with the reviewed studies in terms of pain reduction. Leech therapy decreased the pain by increasing the irritability of cognitive tissues [30]. Another study found that leech therapy reduces pain perception by affecting blood lactate levels [31]. Based on historical experimental studies, several known chemical components in leech salvia have analgesic and anti-inflammatory properties such as hirudin [30,32-34].

The effectiveness of gabapentin in treating neuropathy has been proved in many studies [35,36]. In the present study, the severity and symptoms of neuropathy based on VAS, NSS, and NDS improved some subscales for patients in the control group who used gabapentin over different time intervals, but the conditions in many patients were stable over time. This finding reveals that gabapentin can be considered an effective medication for treating diabetic neuropathy, but based on our findings and other studies which reported controversial findings, it can be replaced with other medication or also traditional medicine such as leech therapy. For instance, Khasbage et al. [37] showed that both duloxetine and gabapentin were effective for the symptomatic relief from diabetic neuropathy and

had similar efficacy, but another study that focused on low-level laser therapy (LLLT) and gabapentin in the management of peripheral neuropathy showed that gabapentin was less effective and it increased some of the liver enzymes such as serum alanine aminotransferase (ALAT), and aspartate aminotransferase (ASAT) in diabetic rats [38]. These findings indicated the effectiveness of traditional medicines over modern therapies in the treatment of neuropathy. This study illustrated that diabetic patients who suffered from neuropathy and used evening primrose oil had lower pain and neuropathy scores versus other patients who used gabapentin with placebo [39]. The present study showed that patients undergoing leech therapy, as traditional medicine, had better conditions compared with others who used gabapentin. More information is needed to clarify the benefits of gabapentin in the treatment of diabetic neuropathy in the presence of traditional medicines.

One of the contributions of this study was confirming the effectiveness of leech therapy on motor nerve function. The present study revealed that patients who were treated leeches in comparison to the patients who were treated with gabapentin had better conditions after 45 days. Although the function of two tibial nerves and DPN improved for the majority of patients after 45 days, the patients exposed to leech therapy in the left tibial amplitude had a better condition. These patients also had better performance in tibial amplitude for both ankles and DPN latency and amplitude over 45 days. Two case reports revealed the role of leech therapy in the treatment of median nerve compression due to forearm hematoma [40,41]. The effect of gabapentin on the functions of motor and sensory nerves was reported in some studies. One study showed that the nerve conduction velocity significantly improved in diabetic patients who used only gabapentin for treating diabetic neuropathy [42]. Based on the results, the NCV improved the amplitude of DPN and latency of tibial nerves in some patients in the gabapentin group over time. These findings confirmed that gabapentin has its beneficial effects as a chemical drug, but leech therapy has a better performance. There is scant evidence and further studies are needed to explain the effectiveness of leeches versus gabapentin in the functions of motor and sensory nerves.

Limitations

This study had some limitations. The first limitation was related to the subjective nature of some outcomes such as pain could have led to an inaccurate estimation of variables. Second, our patients were not blinded. In addition, three patients withdrew from the study; one patient in the leech therapy group in the second time interval and two patients in the gabapentin group in the third time interval. We estimated the missing data of these patients via statistical methods. The last limitation of our study was financial expenses for assessing EMG-NCV for all patients on long-term trends. Therefore, the EMG-NCV was only assessed at baseline and 45 days after the intervention.

Conclusion

Diabetic neuropathy is an important issue in controlling and treating diabetes mellitus which affects all aspects of patients' lives. Leech therapy as a traditional medicine practice has good performance due to hemodilution, segmental counter-irritation, and antinociception. The results of this study showed that using traditional medicines such as leech therapy for diabetic patients was more effective compared to conventional medications like gabapentin. The severity and symptoms of neuropathy were greatly improved in patients treated with leech therapy compared to patients taking gabapentin. The function of two motor nerves in lower limbs revealed that leech therapy can be advised for diabetic patients who suffered from neuropathy. Traditional medicines can be used in combination with conventional medicines. We suggest conducting clinical trials with a large population to clarify the effect of traditional medicines such as leech therapy on diabetic neuropathy in combination with other conventional medicines.

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Conflict of Interests

None.

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