The Effect of Transcutaneous Application of Gaseous CO$_2$ on Diabetic Symmetrical Peripheral Neuropathy—A Double-Blind Randomized Clinical Trial

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Abstract: Aim: Diabetic symmetrical peripheral neuropathy is a common complication of diabetes mellitus. Patients treated with transcutaneous CO$_2$ application for chronic wounds reported an improvement in peripheral sensations. This study aimed to evaluate the effect of transcutaneous application of gaseous CO$_2$ on diabetic symmetrical peripheral neuropathy. Methods: A prospective randomized, double-blind study was performed at the University Medical Center Ljubljana between September 2019 and September 2020. Sixty consecutive patients with diabetes with a unilateral chronic wound were randomized into either a study group that received transcutaneous CO$_2$ therapy or a control group that received placebo treatment with air. Results: Vibration, monofilament sensation, and temperature of the big toe improved significantly in the study group ($p < 0.001$, for vibration sensation, monofilament test and temperature of the big toe), but not in the control group ($p = ns$ for all evaluated outcomes). Conclusion: According to our results, a transcutaneous application of gaseous CO$_2$ shows promising results in treating diabetic symmetrical peripheral neuropathy. Considering the major consequences of sensory loss leading to foot ulceration and possibly amputation, we believe this treatment approach deserves future attention and investigation as a treatment modality of diabetic symmetrical peripheral neuropathy.

Keywords: diabetic peripheral neuropathy; carboxytherapy; CO$_2$ therapy; diabetic foot; transcutaneous CO$_2$ therapy

1. Introduction

Diabetic symmetrical peripheral neuropathy (DSPN) is a well-known complication of diabetes mellitus types 1 and 2 [1–4]. Symptoms may be diverse, depending on the affected nerve fibers [5]. The first symptoms usually include impaired thermal sensation, pain, or unpleasant sensation in the feet, especially at night. As the impairment of nerve fibers progresses, patients typically complain of numbness, tingling, pain, weakness, and hypo- and hyperesthesia; the latter two begin in the feet and spread in a stocking distribution. Symptoms are typically symmetric; however, sensory symptoms are more pronounced than motor symptoms [2–4,6,7]. The major underlying cause of DSPN is hyperglycemia, although dyslipidemia also plays an important role in diabetes mellitus type 2 [7]. Nerve blood flow impaired due to hyperglycemia increases oxidative stress and inflammation, usually first affecting small-diameter sensory nerves responsible for temperature sensation and painless injury [5,6,8]. The impairment of large fiber sensory nerves responsible for kinesthetic senses of joints and vibration is exhibited in decreased ankle reflexes and a loss
of balance while walking [3,4,7]. Patients reporting a loss of sensation are at a higher risk of developing foot ulcers [5].

Maintaining normoglycemia seems to be the only preventive measure to avoid or postpone DSPN. According to clinical trials, enhanced glucose control proved much more effective in type 1 diabetes than type 2 [7]. Despite careful treatment, the probability of developing DSPN over the years is approximately 30% and 50% for diabetes type 1 and type 2, respectively [8].

Current treatment protocols include pain relief with opioids, antidepressants, anticonvulsants and many other medications still being evaluated and investigated [7–10]. Because of limited effectiveness and known adverse effects, non-pharmacological treatment approaches like transcutaneous electrical nerve stimulation and monochromatic infrared energy therapy have been investigated [10–15].

Recently, our group investigated the effect of transcutaneous application of gaseous CO$_2$ on diabetic chronic wound healing [16]. After only a few treatment sessions, patients reported finally sleeping through the night because the burning and tingling sensations in their feet had diminished or had even disappeared, which led us to perform further investigations. As described in our previous article, transcutaneous application of CO$_2$ seems to have an immediate effect on vasodilatation and elevates the oxygen release from Hb via the Bohr effect [16–19]. It seems that, as the therapy is repeated, neangiogenesis is induced [20,21]. Since microvascular dysfunction plays an important role in DSPN, we expected transcutaneous CO$_2$ application to improve sensation in patients with DSPN [6,22]. Therefore, this study aimed to evaluate the effect of transcutaneous application of gaseous CO$_2$ on diabetic symmetrical peripheral neuropathy.

2. Materials and Methods

The research was designed as a prospective randomized, double-blind study performed at the University Medical Center Ljubljana between September 2019 and September 2020 (Clinicaltrial.gov ID: NCT04561609). The study included patients with diabetes with a unilateral chronic wound and symptoms of DSPN with scores above three on the Michigan neuropathy scoring instrument (MNSI) [23]. This study included only patients without previous amputations to fully evaluate the vibration sensation and monofilament test on all typical points. Patients with asymmetrical neuropathy due to other causes were excluded. Additionally, patients with comorbidities where CO$_2$-induced vasodilatation could theoretically worsen their condition (deep vein thrombosis, chronic kidney diseases grade III and IV, chronic heart diseases NYHA III and IV, patients with known malignant diseases, patients with progressive infection, signs of systemic infection with elevated inflammatory markers, or osteomyelitis) and patients with alcohol abuse were also excluded.

Sixty patients who met the defined period’s inclusion criteria were randomized into a study or a control group. The detailed description of the transcutaneous CO$_2$ therapy using a peripheral vascular rehabilitation system (PVR System; Derma Art, Brežice, Slovenia) is described in a previous publication [16]. Patients in the study group received transcutaneous CO$_2$ therapy for 45 min per session every day (from Monday to Friday) for four weeks (20 sessions altogether). Instead of gaseous CO$_2$, the air was used as a placebo treatment on patients in the control group. Before inclusion, patients were familiarized with the study protocol, received all the relevant information on the study from the medical staff, and afterward gave their written informed consent. Both patients and the doctor who evaluated their sensation status before and after the completing treatments were blind for group assignment (study or control). The nurse performing the transcutaneous CO$_2$ therapy used a random number generator to randomize patients into one of the two groups. After completing the study, data were compared for the two groups, which were subsequently disclosed as study or control groups.

The basic patient characteristics are presented in Table 1.
Table 1. Basic characteristics of the study and the control groups.

|                        | Study Group | Control Group | p    |
|------------------------|-------------|---------------|------|
| No. of patients        | 30 (24 M, 6 F) | 30 (24 M, 6 F) | 1.000|
| Average age of patients (years ± SD) | 65.3 ± 11.9  | 66.76 ± 10.6  | 0.640|
| BMI (mean kg/m² ± SD)  | 29.85 ± 5.0  | 30.2 ± 4.9    | 0.756|
| Tobacco smokers yes/no | 29.85 ± 5.0  | 30.2 ± 4.9    | 0.756|
| HbA1c before therapy (mean% ± SD) | 7.08 ± 0.85 | 7.00 ± 0.90   | 0.736|
| Diabetes mellitus type (type 1/type 2) | 1/29       | 2/28          | 0.999|
| Average score on Michigan neuropathy scoring instrument (score ± SD) | 8.23 ± 2.61  | 8.67 ± 2.55  | 0.518|

Legend: BMI, body mass index; F, females; M, males.

The main outcome measure was an improvement of DSPN based on vibration sensation, monofilament test. Additionally, we measured the toe skin temperature.

Vibration sensation (measured before the first and after the last CO₂ or placebo therapies) was performed using a 128 Hz tuning fork on five standard points on each foot (on the skin above the bony prominence of the first metatarsophalangeal joint (MPT), the base of the hallux, malleolus, diaphysis of the tibia, and tibia tuberosity). Results are presented as the number of points or scores (out of ten) with no vibration sensation [24]. Monofilament testing was performed before the first and after the last CO₂ or placebo therapies using Semmes–Weinstein monofilament (SWM) 10 g on eight standard points of each foot (on the plantar surface of the big toe, the third and fifth toes, on the plantar surface of the first, third and fifth metatarsal heads, on the plantar arch and the plantar surface of the calcaneus). Results are presented as the number of points or scores (out of 16) with no sensation [25]. An additional outcome measure was the temperature measured on the plantar aspect of the big toe using an infrared thermometer (Voltcraft IR 260-85, Voltcraft, London, United Kingdom). Temperature measurement was standardized—before the first treatment and before the 20th treatment (to exclude increased temperature during CO₂ therapy due to vasodilatation). Patients were left to lie on the examination table barefoot for 20 min to adjust to the room temperature.

The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (approval ID: KME 84/02/16).

For statistical analysis, a chi-squared test was used for binomial variables (sex, diabetes mellitus type and smoking status), and a paired T-test was performed to compare the continuous variables before and after treatment and between the groups. The mean differences and 95% confidence intervals (95% CI) were calculated with two-sided probability (p) values. The level of significance was set at p < 0.05. Statistical analysis was performed using IBM SPSS Statistics, v. 24 (IBM Corp, Armonk, NY, USA).

3. Results

Table 2 shows the vibration sensation and monofilament test values and the temperature of the big toe before and after CO₂ or placebo treatments. Results show that before the treatment, the mean values of vibration sensation, monofilament test and big toe temperature were comparable between the groups (p vibration ab = 0.841; p monofilament ac = 0.127; p temperature ac = 0.415).

Table 2. Vibration sensation, monofilament test and temperature of the big toe before and after treatment in both legs for the study and control groups (a study group before therapy, b study group after therapy, c control group before therapy, d control group after therapy).

|                        | Study Group (N = 30) | Control Group (N = 30) |
|------------------------|----------------------|------------------------|
|                        | Before CO₂ Therapies a | After CO₂ Therapies b  | Before placebo treatment c | After placebo Treatment d |
| Vibration sensation score | 5.467 ± 2.573        | 2.583 ± 2.173     | 5.333 ± 2.537   | 4.867 ± 2.596          |
| Monofilament test score | 11.700 ± 4.417       | 5.183 ± 3.244     | 10.083 ± 3.618  | 8.917 ± 4.027          |
| Temperature in °C on the big toe | 26.277 ± 2.875 | 30.651 ± 1.611 | 26.882 ± 2.828 | 27.263 ± 2.968 |

Legend: results are presented as (mean ± SD).
Vibration, monofilament sensation, and the big toe temperature improved significantly in the study group (see $p$ vibration $ab$, $p$ monofilament $ab$ and $p$ temperature $ab$), but not in the control group (see $p$ vibration $cd$, $p$ monofilament $cd$ and $p$ temperature $cd$).

After the treatment, the vibration sensation, monofilament test and toe temperature between the groups differed significantly ($p$ vibration $bd = 0.001$; $p$ monofilament $bd < 0.001$; $p$ temperature $bd < 0.001$).

No significant differences were noted in measured parameters between the leg with a chronic wound and the contralateral leg before or after the CO$_2$ therapy. Improvement in vibration sensation, monofilament test, and temperature of the big toe after CO$_2$ therapy was comparable in the leg with a chronic wound and in the contralateral leg without a wound (Figures 1–3). There was no difference in the observed parameters in any leg in the control group (Figures 1–3). Figures 1–3 present monofilament test, vibration sensation and temperature of the big toe before and after CO$_2$ treatment in the study and control groups—a comparison between the leg with a chronic wound and the contralateral leg without a wound.

![Figure 1](image1.png)

**Figure 1.** Monofilament test before and after the treatment. *$p < 0.001$.*

![Figure 2](image2.png)

**Figure 2.** Vibration sensation test before and after the treatment. *$p < 0.001$.*
4. Discussion

Our study showed a positive effect of transcutaneous gaseous CO$_2$ application on feet sensations tested with monofilament and vibration. The vibration sensation and monofilament test were performed before treatment and after four weeks of CO$_2$ therapy or placebo treatment. Before the therapy, patients in both groups had no vibration sensation in five of the ten standard points. The monofilament test showed a loss of sensation in 11 (study group) or ten (placebo group) out of 16 standard points (Table 2). The pretreatment temperature of the big toe was comparable in both groups.

After four weeks of therapy, sensation was significantly improved in the study group ($p < 0.001$ for vibration and monofilament test), while no improvement was noted in the control group. Similarly, the temperature of the big toe was significantly higher in the study group, while there was no difference in the control group. These results are of great clinical importance because neuropathy is a strong cofactor in the etiology of chronic wounds in patients with diabetes and no effective therapy yet exists for this condition [6]. The only remaining approach is enhanced glycemic control, which seems to be effective predominantly in type 1 diabetes [6].

The pathogenesis of DSPN is not completely understood, but it seems that disturbances in blood flow play an important role [26]. The two theories explaining pathogenesis (metabolic and vascular) are obviously both involved in the pathogenesis of DSPN [27]. Metabolic changes are being intensively investigated to understand and treat the condition that affects millions of patients worldwide [6].

The immediate effects of transcutaneous gaseous CO$_2$ application are vasodilatation and elevation of oxygen release from Hb via the Bohr effect. As the therapy is repeated, neoangiogenesis is induced [16,20,21]. There is no simple explanation for the observed positive effect of transcutaneous application of gaseous CO$_2$ on DSPN in patients with diabetes. The improved microcirculation could be the underlying mechanism. The vasodilatory effect in transcutaneous CO$_2$ therapy is mediated via the NO-dependent pathway [28–30]. The role of the NO-dependent pathway in DSPN is not clear. Several studies to date have evaluated the role of endothelium-dependent and endothelium-independent microvascular dysfunction in peripheral neuropathy with no clear explanation of pathogenic pathway so far [6,22,31–35]. The improved microcirculation after transcutaneous CO$_2$ therapy was confirmed in a previous study [36]. In this study, we measured the temperature of the great toe as an additional parameter depending on microcirculation.
The role of natural CO₂-rich water is known for its positive effects on wound healing. Therefore, its medical application has been modified over the past decades. CO₂-enriched water is used more often than gaseous CO₂. The primary goal in our first published research was chronic wound healing, and we decided to use gaseous CO₂ to avoid the moisturizing of the wound and inhaling of evaporated CO₂ from water [16]. Since one of the positive observations was also recovery of sensory loss, we decided to adhere to the same mode of CO₂ application.

According to our knowledge, this is the first double-blind, randomized research investigating the influence of the transcutaneous application of gaseous CO₂ on DSPN. However, a positive effect was previously observed in two publications with patients treated for chronic wounds on feet [37,38].

Shalan et al. included 22 patients with diabetes who immersed their feet in CO₂-enriched water at 37 degrees for 30 min each day for 15 days [37]. The sensation of the area was evaluated on a scale from one (normal sensation) to five (no sensation) using a pressure pin. Results showed improved sensation from 4.36 before CO₂ therapy to 3.45 after therapy. There was no control group, and statistical evaluation is not presented.

Three years later, Abdulhamza et al. used the same application mode as Shalan [38]. One hundred patients with diabetes and chronic wounds immersed their feet in CO₂-enriched water, only this time the treatment protocol was somewhat modified—their feet were immersed for 30 min three times a week for 12 weeks. The control group received standard treatment for chronic wounds. Sensation was evaluated similarly to Shalan’s method, using a pressure pin. The results showed significant improvement in sensation in the CO₂-treated group after the therapies compared to pretreatment values and compared to the values of the control group ($p < 0.01$).

Duration of treatment and repetition of the CO₂ applications seems to be of importance. Abdulhamza achieved statistically significant improvement in sensation as he increased the number of therapies compared to Shalan. In previously mentioned publications, the duration of the therapy is 30 min [37,38]. We decided to extend the exposure time considering our previous experiences. In healthy subjects, the temperature of the skin reached a plateau in 30 min [19]. In patients with diabetes where circulation is impaired, we added 15 min to ensure the desired effect. The number of repetitions was 20 (4 weeks, 5 times a week), which was enough to heal most of the wounds and achieve improvement in the microcirculation [16,19,36]. Considering previous results, we maintained the same protocol to evaluate treatment results for DSPN.

Another non-pharmacological treatment approach, which has been investigated to treat DSPN, is a monochromatic infrared energy therapy. The first publications were promising, but additional research findings did not corroborate previous results [11–14]. A possible explanation stated that patients treated with monochromatic infrared energy therapy also had foot infections that could aggravate the neuropathy [13]. The recovered sensations could have been falsely attributed to monochromatic infrared energy therapy as the infection was treated.

To overcome possible bias, we included only patients with a single unilateral chronic wound with no signs of progressive or systemic infections (no elevated inflammatory parameters) to separately evaluate the monofilament and vibration sensation on wound-affected and unaffected legs. In the PVR System, transcutaneous gaseous CO₂ therapy is performed on the entire lower part of the body, meaning that the affected and unaffected legs were equally treated. Results showed comparable improvements in sensation in both legs in patients treated with gaseous CO₂ (Figures 1 and 2).

Another limitation of our study is the subjectivity of the methods for sensory evaluation since they are based on patients’ responses. The vibration sensation and monofilament tests are recommended for screening and evaluating peripheral neuropathy in diabetic patients [23–25]. To overcome this limitation, the study was designed as a double-blind, randomized trial in which neither the patient nor the doctor evaluating the results did not know the patient’s group. The results of both subjective outcomes—monofilament
test and vibration sensation test were following results of the temperature of the great toe, which was objectively evaluated with an infrared thermometer. Previously confirmed improved microcirculation [36] could represent the underlying mechanism that improves skin temperature and blood supply to the nerves and, therefore, sensory improvement. Despite careful consideration of possible bias, our study is the first one evaluating the effect of transcutaneous gaseous CO₂ application on DSPN and, therefore, needs further evaluation.

5. Conclusions

According to our results, transcutaneous application of gaseous CO₂ shows promise in treating DSPN. There are some reports in existing literature confirming our results. Considering the major consequences of sensory loss leading to foot ulceration and possible amputation, we believe that this treatment approach deserves future attention and investigation as a treatment modality of DSPN.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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