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Assessment of the CO_2 water bath therapy effectiveness on diabetic foot ulcers through VEGF and TNF-α levels

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Abstract

Vasculopathy is typically seen in diabetic patients, and can lead to foot ulcerations. Carbon dioxide (CO_2) therapy was found to improve chronic wound healing in patients with vascular impairment. This type of therapy refers to the transcutaneous and subcutaneous application of CO_2 as well as CO_2 water baths for therapeutic purposes. In the method used herein, artificial CO_2-containing water for foot bathing was generated by dissolving Carbothera® (MRE-SPA-MD; Mitsubishi Rayon Engineering, Tokyo, Japan) and generating CO_2 (free CO_2 concentration: 1,000–1,200 mg/L; pH 4.6). The foot of each patient was immersed in CO_2-enriched water (depth of 20–30 cm, 37–38°C, 30-min duration) three times per week, for the next three months. Dramatic clinical improvement was observed in the CO_2 water bath therapy group before and after the treatment, while both the blood levels of the vascular endothelial growth factor and of the tumour necrosis factor-alpha in these patients exhibited significant changes. The advantages of this method are the absence of pain and the protection against infection, while the improved angiogenesis and oxygenation can result in healing of the chronic wound.

KEYWORDS

diabetic foot ulcer, carbon dioxide therapy, vascular endothelial growth factor, tumour necrosis factor-alpha.

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1. INTRODUCTION

Diabetic foot ulcers (DFUs) are a common consequence of longstanding and poorly managed diabetes. Of the estimated 537 million people worldwide who suffer from diabetes [1], 19–34% will develop a DFU in their lifetime [2]. Approximately 20% of the people who develop a DFU will require amputation that will be either minor (below the ankle) or major (above the ankle) [2], while 10% will die within one year of their first DFU diagnosis [3,4]. The treatment of DFUs is a global major health care problem, resulting in high care costs and mortality rate. The recognition of infection and ischaemia is very important, as it allows us to determine factors that predict the healing progress of the DFU and the risk of amputation. Ischaemia, the lack of blood circulation, develops due to chronic complications of diabetes. This can result in the

development of gangrene of the DFU, which may require amputation if not recognized and treated early. There are a number of DFU classification systems such as the Wagner, the University of Texas, and the SINBAD classification systems, which include require information regarding the site of the DFU, its area, its depth, the presence of neuropathy, the presence of ischaemia, and the presence of infection [5]. This study focuses on ischaemia and infection, which are defined by the presence of poor reperfusion to the foot [6] and the presence of bacterial soft tissue or bone infection in the DFU (as confirmed by blood testing) [7], respectively.

2. PATIENTS AND METHODS

A total of 95 patients with DFU (mean age: 55.6±11.6 years; 59 men, 36 women) were divided into two groups in this randomized double-blind study. The traditional therapy group was treated by using advanced dressings and antibiotics (control group), while the CO_2 therapy group (study group) was treated by using advanced dressings with antibiotics and CO_2 therapy. In brief, artificial CO_2 -containing water for foot bathing was generated by dissolving Carbothera® (MRE-SPA-MD; Mitsubishi Rayon Engineering, Tokyo, Japan) and generating CO_2 (free CO_2 concentration: 1,000–1,200 mg/L; pH 4.6). The foot of each patient was immersed in CO_2 -enriched water (depth of 20–30 cm, 37–38°C, 30-min duration) three times per week, for the next three months.

Written approvals for the undertaking of this study were obtained by the Research Ethics Board at the College of Medicine of Baghdad University and by the Hospital Research Review Committee, prior to the implementation of the study and the enrolment of any participants. Moreover, participants were given verbal information describing the nature of the study.

Blood samples (5 mL) were obtained from the two groups before and after the study. The blood levels of the vascular endothelial growth factor (VEGF) and of the tumour necrosis factor-alpha (TNF-α) were measured by the ELISA method. Moreover, the size, colour, and sensation of the ulcerative area were all evaluated and compared between the two groups.

3. RESULTS

According to the demographic data of the patients, there was no significant age difference between the study group and the control group, and the same applies with regard to their distribution based on their sex. A significant deference in the size, colour, and sensation of the ulcerative region was observed; these parameters were all improved in study group (Figure 1A). According to the undertaken laboratory investigation there was no significant deference between the study group and the control group with regard to their random blood sugar, glycated haemoglobin, renal function test, liver function test, and lipid profiles. However, the VEGF and TNF-α levels revealed highly significant differences when compared before and after the therapy in the study group ($p<0.001$; Figure 1B–C).

4. DISCUSSION

The findings of our study have confirmed that the application of CO_2 in a water bath can significantly improve the healing of DFUs. The wounds in the study group patients (that received CO_2 therapy) healed significantly faster compared with those of the control group. After CO_2 therapy, 66% of the wounds healed completely, as compared to 0% in the control group.

There is limited research on the specific effect of CO_2 therapy on bacterial infections in DFUs. CO_2 is a natural component of the atmosphere and is generally non-toxic to humans. The CO_2-enriched water bath therapy has been reported to accelerate the wound healing process of DFUs by lowering the pH levels of the wounds. As bacteria often thrive in neutral or slightly alkaline conditions, the lower pH inhibits their growth. Moreover, by enhancing the oxygen delivery, the CO_2-enriched water has the ability to enhance the oxygen-carrying capacity of red blood cells, thereby resulting in improved oxygen delivery to the wound. This increased oxygenation can be detrimental to anaerobic bacteria, as they prefer low oxygen or oxygen-deprived environments [8].

Shalan *et al.* [7] have published an observational study on 22 diabetic patients with chronic wounds. Their study had no control group, and they immersed their patients' feet into CO_2 −enriched water once daily, for 30 min, for 15 days. During this time, they noticed an improved blood flow (confirmed by Doppler flowmeter) and an improvement in the wound colour. There was no significant improvement observed in terms of the wound area reduction. The authors [7] rightfully assumed that the CO_2 treatment would have probably improved wound healing if the treatment lasted longer. Three years later, Abdulhamza *et al.* [8] conducted a study by using the same device as Shalan *et al.* [7].

Abdulhamza *et al.* [8] have performed a study similar to ours, with the exception that theirs was not double-blind. They included 100 diabetic pa-

tients with chronic wounds, divided into study and control groups. The study group received both standard treatment and CO_2 therapy. The latter was administered as gas in the water bath as in our study. The patients immersed their feet in CO_2-enriched water for 30 min, three times per week, for three months. After the treatment, a significant improvement was seen in the study group through Doppler imaging of the large leg arteries and veins, and a decrease in the wound size was also noted. The control group exhibited no differences in the observed parameters when assessed before and after treatment.

It is well-established that the CO_2 therapy exerts a vasodilatory effect, probably influenced by a NO-dependent pathway. Minamiyama and Yamamoto [9] have used intravital microscopy in order to confirm the subcutaneous vasodilatation occurring after CO_2 therapy in rats. Another mechanism that is involved immediately after the CO_2 application is the Bohr effect, which was confirmed by Sakai *et al.* [10]. The Bohr effect means that in increased pCO_2 changes the haemoglobin (Hb) – oxygen (O_2) dissociation curve shifts to the right, and so the Hb releases more O_2 and the tissue gets more oxygenized. It has already been shown that even 20 min of a foot's immersion to CO_2 can improve the tissue oxygenation. Sakai *et al.* [10] also verified this effect *in vivo* (in seven healthy volunteers) and confirmed the change between oxygenated and deoxygenated Hb during single transcutaneous applications of dry CO_2 therapy [10]. Therefore, a single treatment with CO_2 exerts vasodilatation and elevates O_2 release from Hb via the Bohr effect [10]. In order to enhance wound healing, the CO_2 application must be repeated so as to maintain tissue supply with O_2 ad to induce neo-angiogenesis; a delayed CO_2 effect of the CO_2 therapy. The importance of the repetition of the CO_2 therapy can be observed from the comparison of the findings of the two aforementioned studies on diabetic patients [7,8].

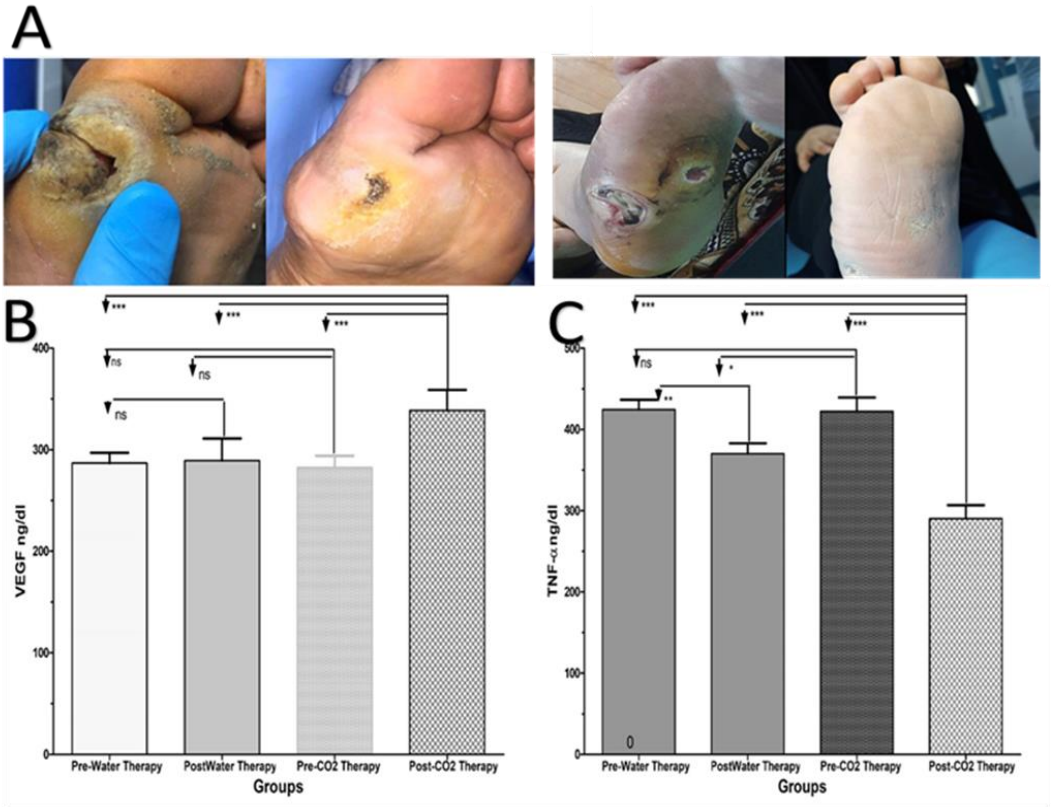

Figure 1. (A): Examples of diabetic foot ulcer (DFU) wounds before and after the CO_2 therapies in the study group. (B): Mean vascular endothelial growth factor (VEGF) blood levels before and after therapy in the two groups of the study. (C): Mean tumour necrosis factor-alpha (TNF-α) blood levels before and after therapy in the two groups of the study. Note: values in the graphs represent means ± standard deviations. Statistical significance: *, $p<0.05$; **, $p< 0.01$; ***, $p<0.001$; ns: non-significant.

The strength of our study lies in its methodology, as it was designed as a controlled double-blind randomized study. The limitations of this study are the differences in the wound size and volume, as well as their response to the number of exposure sessions to CO_2 therapy, which may differ from patient to patient. However, the limitations were minimized by applying more sessions to those patients. After considering our results and the effects of the CO_2 therapy described in the literature, one can safely conclude that the CO_2-enriched water bath could be an effective adjuvant therapy in the treatment of chronic wounds. The latter are sometimes very difficult to treat; therefore, it is important to know what kind of adjuvant therapy is available and confirmed as effective. Since the mode of action of the CO_2 therapy involves the improvement of vascularization, it can be combined with previously described advanced therapeutic approaches in order to treat even the most persistent wounds.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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