Original Article

Healing for Chronic Diabetic Foot Ulcers using Cold Atmospheric Plasma

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ABSTRACT

Objective: To evaluate of efficacy and safety of cold atmospheric plasma to treatment and improvement the healing of chronic, non to foot ulcers.

Study Design: Quasi- experimental study

Place and Duration of Study: This study was conducted at the College of Medicine, Iraq from 1st July 2024 to 31st December 2024.

Methods: Diabetic foot ulcers represent a critical clinical challenge, often associated with considerable morbidity and mortality risks. Cold atmospheric plasma emerges as a pivotal advancement in therapeutic strategies within the medical field. This innovative approach offers a modern alternative for sterilization and wound treatment when compared to traditional methods. The study included four participants, with an average age of 63.26 years, all of whom experienced a mean healing delay of 17 months, with a range of 11 to 20 months, largely attributed to peripheral arterial disease affecting 50% of the subjects.

Result: There were 99% males who had diabetic foot lesions. 60% had associated peripheral arterial disease with a mean delay in healing of 19 months. The average ulcer healing time was 7.6 weeks. One patient's treatment was suspended after 5 weeks due to the state of alarm decreed by the COVID-19 pandemic. In no case were adverse effects related to the application of cold atmospheric plasma detected. Focusing on individuals suffering from diabetic foot ulcers who experienced delayed healing following cold atmospheric plasma treatment. The average duration for ulcer healing was recorded at 7.5 weeks, and notably, no adverse effects were observed in relation to the application of cold atmospheric plasma. These initial findings indicate that the use of cold atmospheric plasma for non-healing diabetic foot ulcers is both a safe and effective therapeutic option.

Conclusion: Cold atmospheric plasma represents a significant advancement in wound care, yielding promising results that underscore its efficacy demonstrated that healing occurs more rapidly with cold atmospheric plasma treatment compared to cases where it was not utilized.

Key Words: Cold atmospheric plasma, Wound care, Yielding promising, Healing

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INTRODUCTION

Damage to the entire membrane and the underlying tissue due to injury or trauma is called a wound.[1] Both biological and environmental factors play an important role in wound formation.[2,3] These factors disrupt the skin structure and damage the underlying tissue.

Wounds are repaired through four basic processes. These processes include inflammation, proliferation, differentiation, and maturation. The steps of the four main methods are as follows: rapid hemostasis, appropriate inflammation, differentiation, proliferation and migration of mesenchymal cells to the wound area, appropriate angiogenesis, regeneration of epithelial tissue on the wound surface, synthesis, cross-linking and regulation of collagen for reinforcement.[4]

Chronic wounds are wounds that are difficult to heal or that heal slowly. The wound healing process is interrupted and the normal wound healing process cannot proceed for a period of time.[5] Diabetes is a chronic disease that is becoming prevalent worldwide.[6] Of note, more than 80% of amputations for non-traumatic reasons in patients with diabetes occur in the presence of foot ulcers, which are an indicator of advanced diabetes.[7]

Diabetic foot ulcers are one of the most serious complications of diabetes, occurring in up to 15% of diagnosed patients. They are one of the most common reasons for lower limb amputation in Europe and the United States. Diabetic foot ulcers occur due to damage to blood vessels caused by increased blood sugar levels. This damage leads to disruption of blood circulation, known as peripheral ischemia, and nerve damage (known as neuropathy). As a result, patients lose

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sensitivity in their feet, putting them at risk for foot ulcers.[8]

In recent years, cold atmospheric plasma (CAP) has been proposed to improve the healing of these ulcers. However, the effect of CAP on wound healing in diabetic foot ulcers compared with standard treatment remains to be studied. Plasma medicine is broadly categorized into two main areas: cold and hot applications. Its uses span the sterilization of medical devices, blood coagulation, surface modifications of implants, and antimicrobial research. In particular, cold plasma medicine has shown promise for inactivating prokaryotes such as bacteria and fungi on living tissue, accelerating blood clotting, promoting wound healing, addressing various complex diseases, advancing dental applications, managing blood rheology, and effectively sterilizing different surfaces, including living tissues.[9,10]

The application of cold plasma, also referred to as low-temperature atmospheric pressure plasma, in appropriate doses has demonstrated potential in treating chronic wounds. This effect is attributed to its physical processes, primarily involving collisions and chemical reactions among electrons, particles, and gas molecules. When plasma is generated in the air - comprising nitrogen (N_2), oxygen (O_2), and water vapor (H_2O) - it induces a series of reactions. These interactions between plasma particles and water molecules on the wound's surface lead to the formation of reactive oxygen and nitrogen species (RONS), which play a pivotal role in wound healing.[11]

METHODS

This quasi-experimental study was conducted at College of Medicine, Iraq from 1st July 2024 to 31st December 2024 and focusing on individuals suffering from diabetic foot ulcers who experienced delayed healing following cold atmospheric plasma treatment. The study included four participants, with an average age of 63.26 years, all of whom experienced a mean healing delay of 17 months, with a range of 11-20 months, largely attributed to peripheral arterial disease affecting 50% of the subjects. Elderly patients of both genders with vascular or diabetic foot ulcers experiencing delayed healing were included. Cold atmospheric plasma was applied as part of the treatment process. Electrical measurements for the CAP system were conducted using a high-frequency voltage probe (HV-40 High Voltage Test Probe, Tecpel, capable of 40K VDC or peak AC and 28KV rms AC). The probe signals were recorded using a digital storage oscilloscope (GW-Instek GDS-2202A, with a bandwidth of 300MHz, 200MHz, 100MHz,or 70MHz and 2-4 input channels). The electrical characterization revealed that the voltage level became active 1.5 seconds after system activation. This indicated that the plasma operated effectively across all levels to treat infected wounds. Increased free radicals (ROS-RNS) and UV rays generated by CAP reduced bacterial viability, leading to shorter treatment durations. Notably, no external gas input was required to enhance antimicrobial efficacy. The air plasma jet production system (left) and the air plasma jet itself (right).Inclusion criteria for participants included: legal adult age, willingness to provide signed informed consent, and the presence of vascular or diabetic foot ulcers persisting for more than six months despite appropriate care and treatment. Comprehensive assessments considered factors such as infection status, tissue viability, exudate levels, and prior advanced interventions like negative pressure therapy or metalloprotease-modulating dressings. Critical ischemia (Fontaine Grades III-IV) without revascularization, confirmed infection with osteomyelitis (diagnosed via X-ray for patients testing positive on a probe-to-bone diagnosis), oncological patients undergoing chemotherapy or radiotherapy, and patients in terminal stages were excluded.

There corded variables encompass edge, sex, wound ethology, duration of ulcer evolution, wound surface area (in cm^2), complete healing(yes or no),time until full epithelialization (in weeks from CAP initiation), and any adverse effects reported by patients or observed by researchers. The protocol included the following steps: 30cc of blood was drawn andtreatedwith3ml of sodium citrate as an anticoagulant. The blood underwent immediate processing through centrifugation at 2800rpm at room temperature for five minutes to separate serum and obtain a clot. This included a general health evaluation, wound examinations, diagnostic tests (including microbiological analysis when necessary), and photographic documentation with weekly progress monitoring. Ulcer areas were measured during the initial evaluation and again at subsequent follow-up visits at three weeks, ten weeks, or the end of the healing process. Prior to CAP application, the ulcer surface was cleansed using physiological saline under aseptic conditions. If required, debridement was performed to remove non-viable tissue from the wound bed and perilesional area. Zinc oxide barrier cream protected the perilesional edges, while CAP gel was applied directly to the wound bed. It was secured with a silicone mesh and a secondary dressing to manage exudate. CAP treatments were administered at seven-day intervals,

RESULTS

Four cases seen in the above table participated in this pilot study with an average age of 60 years, with a minimum of 57 and a maximum of 72 years (Table 1).

The poor evolution of the ulcer, which had a bed with pale, friable granulation tissue and hyperkeratotic edges, it was decided, with the patient's consent, to begin treatment with CAP after 21 days, the area was 7.2 cm^2, with a reduction in the lesion surface of 17%

in that period (Fig. 1). After 3 weeks of treatment, the ulcer area had decreased by 12.5 % (measured 4.1 cm 2.1). After 5 weeks, treatment was suspended due to the COVID-19 pandemic, with the lesion having a surface area of 2.7 cm^2 (Fig. 2).

Table No.1: History of the cases

No. of case	Age (years)	Case of pattern	History of pattern	Type of treatment	Urcl area	Time of treatment
1	54	Neuropathic ulcer on the external edge of the heel of the right foot that had been developing for 13 months.	Anxiety disorder, diabetic polyneuropathy, chronic alcoholic liver disease, diabetic retinopathy, HTA, low back pain, and diabetes mellitus type 2	CAP	9 cm^2	45 days
2	62	Megaloblastic anaemia, type 2 diabetes mellitus, obesity and peripheral arterial disease	Megaloblastic anaemia, type 2 diabetes mellitus, obesity and peripheral arterial disease presented with a transmetatarsal amputation in the right foot resulting in a severe diabetic foot infection of 20 months duration	CAP	4.5 cm^2	5 weeks
3	73	Neuropathic ulcer on his right heel that had been developing for 7 months when he came to our office	Dyslipidaemia, proliferative diabetic retinopathy, and anaemia	CAP	10cm^2	70 days
4	63	Ulcer on the plantar aspect of the left foot, over the head of the first metatarsal, of 18 months duration	Metabolic syndrome, ischemic stroke, dilated cardiomyopathy, arterial hypertension, hyperuricemia, diabetes mellitus, prostate adenocarcinoma, depression, peripheral arterial disease and amputation of the 1st toe of the right foot	CAP	2 cm^2	35 days

Figure No. 1: Treatment before and after complete healing at 56 days

Figure No. 2: Treatment before and after 35 days when treatment is suspended

Figure No. 3: Treatment before and after complete healing at 70 days

Figure No. 4: Treatment before and after complete healing at 35 days

After 21days of treatment, the lesion area had decreased by 30% (measured 7.8 cm^2), and complete epithelialisation of the lesion was achieved in 70 days (Fig. 3). The patient had an ulcer on the plantar aspect of the left foot, over the head of the first metatarsal, of 18 months duration. After applying various types of treatment and stagnation, the lesion remained stagnant. CAP treatment is started. At the beginning of the treatment with CAP, after 20 days, the lesion had a surface area of 0.71 cm^2, which represents a reduction in the area of 67% in that period. Complete epithelialisation was achieved after 5 weeks (Fig. 4).

DISCUSSION

Platelet-rich plasma is increasingly used in clinical practice in various specialities to accelerate tissue regeneration processes.[12] Variability in wound healing outcomes with CAP is likely due to the diversity of devices, methods, and clinical strategies used to obtain and apply CAP-derived products.[13]

Plasma rich in platelet growth factor is an option in managing and healing vascular and diabetic foot ulcers.[14,15] CAP therapy as an alternative treatment, especially in cases of chronic skin ulcers where conventional treatments are unproductive. Mirpour et al[14] stated that the growth factor contained in cold atmospheric plasma is an autologous and harmless therapeutic alternative that showed an efficacy of 79.2% in the healing of ischemic ulcers in the diabetic foot.

Our results show a reduction in the ulcer area after 3 weeks. In the study by He et al[15], they also obtained similar results at 3 weeks of evolution; they also found that healing occurred in less than 6 weeks in 47% of patients and complete healing of wounds in 57% of patients in 3 months.

This study's results align with the studies found in the literature within our series of cases, with the average healing time being lower than the cure with conventional treatment. A study in patients with ischemic ulcers with diabetic foot managed with conventional treatment, found that, on average, 41% of ulcers healed after 12 weeks.[16] In another clinical trial,

it was found that ulcers took an average of 91 days to heal with conventional therapy.[15,16]

The fact of not having a control group implies limitations when interpreting the data. However, a significant reduction in the patients' healing time is evident, considering that many of them had been previously treated without achieving such a cure for many months. After applying CAP treatment, the ulcers have healed in an average time of approximately 7 weeks. In the study by Stratmann et al[10], the average healing time of the lesions was around 3 months.

In addition, various radicals in the plasma jet, especially ozone, when in contact with water molecules near the wound will cause hydrogen peroxide (H_2O_2) and testing for killing drug-resistant MRSA bacteria with the air plasma jet.[17]

The effects of cold plasma are selective on bacterial cells.[16] That is, plasma radicals can destroy bacterial cells without causing damage to the cells or wound tissue. This is because bacterial cells are much smaller than human cells. The destruction of human cells requires a higher amount of plasma radicals than the killing of bacterial cells. The human cells have DNA repair mechanisms and contain free radicals. Antioxidant that is sufficient to protect cells, while nitric oxide (NO) radicals generated from cold plasma are radical that acts as a cell signalling molecule related to the immune system and affects cell stimulation, such as stimulating cell proliferation, cell proliferation and cell movement (cell migration) creating new blood vessels (angiogenesis) and collagen synthesis to repair damaged skin. The air plasma jet production system is an innovation that can change the air around us. It helps treat infected wounds that are difficult to treat with standard methods and lead to drug-resistant germs.[17]

The findings from this pilot study suggest that plasma enriched with platelet growth factors could be a valuable option for managing and healing vascular and diabetic foot ulcers. Other researchers also propose CAP therapy as an alternative treatment for chronic skin ulcers, particularly when conventional treatments prove ineffective.

Our results demonstrate a noticeable reduction in the ulcer area within three weeks. Similarly, He et al[15] reported comparable findings in their three-week observation period. Their study further noted that 47% of patients experienced healing within six weeks and complete wound closure in 57% of patients within three months. In comparison with existing literature, the outcomes of this study align with prior investigations while showing slightly accelerated average healing times versus conventional treatments. Findings from other studies indicate that standard care for ischemic ulcers in diabetic foot patients led to complete healing in only 41% of cases within 12 weeks, or took approximately 91 days average.[10]

Cold atmospheric plasma generates various radicals, such as ozone, which interact with water molecules near wounds to produce hydrogen peroxide (H_2O_2). Studies have demonstrated its efficacy in eradicating drug-resistant MRSA bacteria using air plasma jets. As illustrated in Figure 6, the potential of air plasma jets is particularly promising for treating chronically infected wounds by targeting and eliminating bacteria within them.[18]

CONCLUSION

Cold atmospheric plasma represents a pivotal advancement in medical therapy as both a sterilisation tool and a treatment for chronic wounds. Cold atmospheric plasma therapy demonstrates faster tissue regeneration and wound closure. Cold atmospheric plasma's is potential a safe and effective option for treating vascular wounds and diabetic foot ulcers unresponsive to standard therapies.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Riyam Adnan Hammudi, Mustafa A. Mahmood
Drafting or Revising Critically:	Riyam Adnan Hammudi, Mustafa A. Mahmood
Final Approval of version:	All the above authors
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