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Cold Atmospheric Plasma in Chronic Diabetic Foot Ulcers

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Abstract: Diabetic foot ulcers are a significant clinical problem with a high risk of morbidity and mortality. The clinical approach of these patients should be urgently evaluated in a multidisciplinary manner by a well-equipped team, the underlying factors causing the ulcer should be revealed, and these factors should be corrected in addition to general wound care principles. Cold atmospheric plasma is an essential step towards new therapeutic methods in medicine. Plasma is a new tool for sterilisation and treatment of wounds compared to conventional treatment methods. From September 2019 to March 2021, the pilot study was carried out at the Baghdad Medical College, Iraq. Individuals with diabetic foot ulcers who had Cold Atmospheric Plasma (CAP) treatment, and a delayed healing period were part of the study. Four patients, with an average age of 63.26 years, participated in the pilot study. A mean healing delay of 17 months, ranging from 11 to 20 months, was associated with peripheral arterial disease, which affected 50% of the individuals. The healing of ulcers took an average of 7.5 weeks. In all cases, no adverse effects were linked to the application of CAP. These preliminary results suggest that applying CAP to non-healing diabetic foot wounds is a safe and effective treatment option. It is concluded that using CAP in treating wounds is advanced and very promising, presenting satisfactory results that demonstrate its effectiveness. It has been shown that with its use, healing occurs more quickly compared to cases in which it was not applied.

Keywords: Diabetic feet, ulcers, plasma, MRSA bacteria, case study.

1 Introduction

Damage to the entire membrane and underlying tissues due to injury or trauma is called a wound [1]. Both biological and environmental factors play an essential role in wound formation. Examples of these, Metabolic diseases, tumours, drug side effects and ecological traumas are given. Due to these factors, the skin's structure is disrupted, and damage occurs to the underlying tissues [2].

These causes of wounds are so common that wound healing is one of human life's most complex biological processes. Under normal conditions, the wound is repaired through four basic processes [3]. These processes include inflammation, proliferation, differentiation and maturation. The steps that occur in the four primary methods are as follows: rapid hemostasis, appropriate inflammation, mesenchymal cell differentiation, proliferation and migration to the wound area, proper angiogenesis, regeneration of epithelial tissue on the wound surface, synthesis, cross-linking and regulation of collagen to provide strength to the healing tissue [4].

Chronic wounds are wounds that are difficult to heal or heal more slowly. For some time, because the wound-healing process is interrupted, a normal wound-healing process cannot be carried out. Usually, it should be cured and cannot determine a definite [5], Diabetes is a chronic pathology that is giving rise to an epidemic worldwide. The prevalence of this pathology has increased more rapidly in low- and middle-income countries [6]. It should be noted that more than 80% of all amputations performed for non-traumatic reasons in people with diabetes are preceded by foot ulcers, which are one of the indicators of advanced diabetes [7].

Chronic wounds (CW) constitute one of the most critical healthcare problems that affect the health system due to the decrease in the quality of life of those who suffer from it and their caregivers, their high economic cost and the dedication of a lot of time by those who suffer from it—nursing professionals [8]. The healing of these wounds is a complex phenomenon, in many cases not fully known, which requires specific knowledge and the work of a multidisciplinary team to be able to cover all aspects and needs.

In diabetic foot syndrome, ulcers, wounds due to metabolic diseases, acute wounds, infected wounds and age-related surgical wounds, the healing process does not proceed in the above order and severe health problems may be encountered [8]. Non-healing wounds are one of the most important clinical problems today. Approximately 60 million people worldwide are treated for non-healing wounds annually [9]. This number is increasing day by day due to the inadequacy and unsuitability of the methods and systems used in wound healing. The most basic

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shortcomings of the methods used today are; The main problems of these methods are that they are slow, painful, cause damage to healthy tissues, take an extended hospital environment, take time to heal, and cannot be used near the eye or mouth area.

Diabetic foot ulcers are one of the most significant complications of diabetes and occur in up to 15% of diagnosed patients, being one of the most common causes of lower extremity amputation in Europe and the United States [10]. The risk of amputation is related to the complex pathophysiology of neuropathy and ischemia in the diabetic foot, which makes it impossible for the foot to remain intact under the stress of daily life [10-11].

Diabetic foot ulcers are produced due to the suffering of blood vessels due to increased blood glucose levels. This damage generates a failure in blood circulation, that is, peripheral ischemia and nerve damage, called neuropathy, through which the patient loses sensitivity in the feet, with the consequent risk of an ulcer occurring in the foot [10]. Because of this loss of foot sensitivity, the diabetic individual will have more incredible difficulty noticing minor foot injuries, such as chafing. These types of conditions can become complicated when the individual does not see them and lead to the foot becoming infected, which can cause ulcers and increase the chances of amputation. Therefore, diabetic foot ulcers are a common complication of diabetes and require specialised treatment. In recent years, cold atmospheric plasma (CAP) has been proposed to improve the healing of these ulcers. However, the effect of CAP compared with standard treatment on wound healing in diabetic foot ulcers remains to be studied [12-13].

New areas and methods are being focused on to overcome these and similar problems. As a current example, plasma applications, the fourth state of matter, in medicine are given [12-13]. Plasma medicine is divided into two areas: cold and hot application areas. It is used in sterilisation of medical devices, blood coagulation, implant surface modification, cancer therapy, and antimicrobial studies [14]. Especially in the field of cold plasma medicine, inactivation of prokaryotes, including bacteria and fungi in living tissue; acceleration of blood clotting, wound healing treatment, cancer treatment; treatment of multiple complex diseases; dental applications; controlling the rheology of blood, effective sterilisation of various surfaces including living tissue [15].

The application of cold plasma or low-temperature atmospheric pressure plasma at appropriate doses has been shown to have potential in treating chronic wounds [15]. This is due to physical processes, especially collisions and chemical reactions between electrons and particles or between particles. This occurs in the plasma produced in the air, which contains nitrogen (N2), oxygen (O2) and water vapor (H2O) gas molecules as components. The chemical reaction between the particles in the plasma and the water molecules on the wound causes the formation of reactive oxygen and nitrogen species (RONS).

A prospective quasi-experimental study has been designed and planned to evaluate the safety and efficacy of CAP for treating vascular ulcers of the lower limbs and diabetic foot with delayed healing. Through this pilot study, the feasibility of said study and the suitability of the action protocol, the variables collected, and the measurement instrument have been assessed.

2 Materials and Methods

This pilot study was carried out between September 2019 and March 2021 in the Vascular Surgery Service University Hospital in Baghdad. Elderly patients of both sexes with vascular or diabetic foot ulcers who were delayed in the healing process and to whom CAP was applied (Figure 1) participated. Electrical measurements for cold atmospheric plasma were performed using a high-frequency voltage probe (HV-40 High Voltage Test Probe, Tecpel, 40K VDC or peak AC or 28KV rms AC). Signals obtained from the probe were acquired and recorded using the oscilloscope (GW-Instek GDS- 2202A Digital Storage, Oscilloscope 300MHz / 200MHz / 100MHz / 70MHz Bandwidth, 2 or 4 Input Channels).

According to the electrical characterisation results, the voltage level becomes active 1.5 seconds after the system starts. This indicator shows that plasma works at all levels, from the lowest to the highest level, to treat infected wounds. Increased free radicals (ROS-RNS) and UV rays reduce the viability of bacteria. This reduces treatment time. There is no need for external gas input to increase antimicrobial effectiveness.

Fig. 1: (left) Air plasma jet production system (right) Air plasma jet

The inclusion criteria were: patients of legal age, of both genders, who had given their consent to participate in the study and who had vascular or diabetic foot ulcers of more than 6 months of evolution and which did not heal despite having received an appropriate approach based on the comprehensive assessment of the patient's care needs and the characteristics of the lesions: presence or absence of

infection, tissue viability, degree of exudate and need for shocks or advanced techniques such as negative pressure therapy or metalloprotease modulating dressing.

The exclusion criteria were: patients with critical ischemia (Fontaine Grades III-IV) who were not revascularised, presence of infection and osteomyelitis (an x-ray was performed on all patients with a positive Probe to bone), oncological patients (in treatment with chemotherapy or radiotherapy) and terminal patients.

The variables collected were: age, sex, etiology of the wound, time of evolution, the surface of the wound (in cm2), healing (yes or no), time (in weeks) until complete epithelialisation from the start of the application of CAP and adverse effects reported by the patient or detected by the researchers. The authors of this article carried out both patient recruitment, data collection, and treatment application. The action protocol followed in this study was: Blood extraction. 30cc of blood is extracted with 3ml of sodium citrate as an anticoagulant. Separation of serum and obtaining the clot: The blood is processed immediately, centrifuging at 2800 rpm in a centrifuge at room temperature for 5 minutes.

The protocol followed in this study consists of a general assessment of the patient, wound examination, instrumental diagnostic tests, microbiological if necessary, and acquisition of photographic images with weekly monitoring.

Before the application of CAP, the area of the ulcers was measured in the first evaluation, which was repeated in each of the visits, comparing these areas at the beginning of the treatment, at 3 weeks and at 10 weeks or at the end of the healing

Disposable paper rulers were used to measuring the ulcers, measuring the length and width in centimetres and calculating the area (length x width) in cm2. To avoid variability, the ulcers were measured following the standard method described by Benskin (2018) [15].

Before applying CAP on the surface of the ulcers, the surface was washed with physiological saline using an asepsis technique and, if necessary, debridement was performed to remove non-viable tissue from the bed and perilesional edges.

Subsequently, the perilesional edges were protected with zinc oxide barrier cream, and the CAP gel was applied, adapted to the lesion bed, and secured with a silicone mesh and a secondary dressing for exudate management. The CAP application was carried out every 7 days.

This study was carried out based on the principles of the Declaration of Helsinki and current national legislation. Signed informed consent was requested from all participants. Verbal information about the treatment and procedure was provided to each patient to achieve maximum patient collaboration in positioning and performing the procedure.

3 Results

Four patients participated in this pilot study with an average age of 62.25 years, with a minimum of 56 and a maximum of 71 years. 100% were men who had diabetic foot lesions. 50% had associated peripheral arterial disease, with a mean delay in healing of 17 months with an interval of 12-20. The average ulcer healing time was 7.6 weeks with an interval of 5-10. 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 CAP detected.

Case 1: A 56-year-old male patient with a history of anxiety disorder, diabetic polyneuropathy, chronic alcoholic liver disease, diabetic retinopathy, HTA, low back pain, and diabetes mellitus type 2.

The patient had a neuropathic ulcer on the external edge of the heel of the right foot that had been developing for 12 months. Given 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. A weekly cure is scheduled, and the established cure protocol for this technique is applied.

At the beginning of treatment, the ulcer area was 9 cm2; after 3 weeks, the area was 7.5 cm2, with a reduction in the lesion surface of 17% in that period. Complete epithelialisation of the lesion is achieved in 8 weeks. Figure 2 shows the images at the beginning of treatment and after complete healing.

Fig. 2: Images at the beginning of treatment and after complete healing at 8 weeks

Case 2: A 62-year-old male patient with a history of 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. Given the delay in healing, it is decided, with the patient's consent, to begin treatment with CAP.

At the beginning of treatment, the lesion area was 4.5 cm2; after 3 weeks of treatment, the ulcer area had decreased by 12% (measured 4 cm2). After 5 weeks, treatment was suspended due to the COVID-19 pandemic, with the lesion having a surface area of 2.5 cm2. Figure 3 shows the images at the beginning of treatment and after 5 weeks

when treatment is suspended.

Fig. 3: Images at the beginning of treatment and after 5 weeks when treatment is suspended

Case 3: A 71-year-old male patient with a history of dyslipidaemia, proliferative diabetic retinopathy, and anaemia. He had a neuropathic ulcer on his right heel that had been developing for 7 months when he came to our office. After 17 months of follow-up in which various types of treatment were performed without achieving a favourable evolution of the lesion, it was decided to start therapy with CAP.

At the beginning of treatment, the lesion had an area of 10cm2; after 3 weeks of treatment, the lesion area had decreased by 30% (measured 7cm2), and complete epithelialisation of the lesion was achieved in 10 weeks. Figure 4 shows the images at the beginning of treatment and after complete healing.

Fig. 4: Images at the beginning of treatment and after complete healing at 10 weeks

Case 4: A 60-year-old male patient with a history of 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.

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, the area of the ulcer was 2 cm2; after 3 weeks, the lesion had a surface area of 0.70 cm2, which represents a reduction in the area of 65% in that period. Complete epithelialisation was achieved after 5 weeks.

Figure 5 shows the images at the beginning of treatment and after complete healing at 5 weeks.

Fig. 5: Images at the beginning of treatment and after complete healing at 5 weeks

No systemic or wound site side effects were observed. Occasionally, maceration was observed around the perilesional skin.

4 Discussion

Platelet-rich plasma is increasingly used in clinical practice in various specialities to accelerate tissue regeneration processes [16]. However, since there are multiple methods of obtaining plasma, the preparations vary in the concentrations of platelets and the consistency of the resulting gel. Several authors have stated that much of the reported 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 [17].

The results obtained in this pilot study led us to consider that plasma rich in platelet growth factor is an option in managing and healing vascular and diabetic foot ulcers. Other authors [18-19] also propose CAP therapy as an alternative treatment, especially in cases of chronic skin ulcers where conventional treatments are unproductive. The study by Mirpour et al (2020) [18] concludes 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 (2020) [20], 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 [21]. In another clinical trial, it was found that ulcers took an average of 91 days to heal with conventional therapy [20-21].

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 (2020) [22], 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 (H2O2) and testing for killing drug-resistant MRSA bacteria with the air plasma jet [23]. As shown in Figure 6, the potential of the air plasma jet is shown to help treat chronically infected wounds by killing bacteria in the wound.

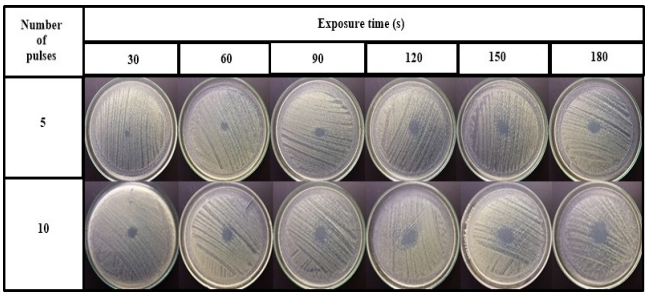

Fig. 6: Effect of an air plasma jet on the killing of drug-resistant MRSA bacteria.

In addition, the effects of cold plasma are selective on bacterial cells [20]. 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 [23]. In addition, 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 It is a 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.

5 Conclusion

Cold atmospheric plasma is an essential step towards new therapeutic methods in medicine. Plasma is a new tool for sterilisation and treatment of wounds compared to conventional treatment methods. The results of this pilot study show the feasibility and relevance of the research study planned to assess CAP's capacity to regenerate tissue and achieve a reduction in the time necessary for complete epithelialisation and closure of vascular wounds and refractory diabetic foot to conventional treatments. According to these preliminary results, applying CAP to the bed of non-healing vascular and diabetic foot wounds is a safe and effective therapeutic option. It would be necessary to carry out clinical trials with a control group to verify the effectiveness, safety, and efficiency of the application of CAP in all types of chronic wounds and compare the results of said therapy with those obtained through conventional therapies.

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