Advances in Bioresearch
Adv. Biores., Vol 14 (2) May 2023: 110-118
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.3.110118

Advances in Bioresearch

ORIGINAL ARTICLE

Combination of Photodynamic Therapy and Multi-Wavelengths Photobiomodulation in Chronic Wound Healing Disorders: Case series

Michael Weber*[1], Aref Ghanbarzadeh[2], Yasaman Zandi Mehran[3],Hans Michael Weber[4], SeyedMohammad Hosseini Kazerouni[5], ,Fatemeh Zandi Mehran[6],SeyedehParnian Hosseini Kazerouni[7],Amirreza Amjadi[6], Seyed Ali Golestanha[8], Nahid Rahbar[9], Kamran Mansouri[10]

[1] Head of European Laser and TCM Laser Academy, Germany
[2] Head of Central Ajudanye Clinic, Tehran, Iran
[3] PhD of Biomedical Engineering, Assistant Professor, IAU, Tehran, Iran
[4]Head of International Society of Medical LASER Applications, Lauenfoerde, Germany
[5]Head of Kourosh Health and Diabetes Clinic, Isfahan, Iran
[6]Kourosh Health and Diabetes Clinic, Isfahan, Iran
[7]Medical director of Kourosh Health and Diabetes Clinic, Isfahan, Iran
[8] Head of Radiology Department, Shahed University, Tehran, Iran
[9] Medical director of wound department, European LASER Clinic, Dubai, UAE
[10]Medical Biology Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran
***Corresponding Author: Dr. med. Michael Weber**

ABSTRACT

Diabetes has a dramatic impact on global health. Diabetes mortality has increased in recent years, because of a new century lifestyle in many countries. In addition, many people die from diabetic wound infections. Therefore, there is still a need for therapies to help people with these chronic wounds. The objective of this study was to evaluate if Photodynamic Therapy (PDT) with Riboflavin and a multi wavelengths laser therapy would be able to treat resistance chronic diabetes wounds to assess whether patients could avoid amputation. The study consisted of 5 diabetic patients, each of whom underwent of PDT and multi-wavelengths Photobiomodulation (PBM). All patients had type 2 diabetes and resistant chronic diabetic wounds that were decided for amputation and surgery by a medical committee. Riboflavin was applied to the wound and irradiated with three wavelengths of violet laser 375 nm, blue laser 447 nm, at 10J/cm^2. A 630 nm red laser and a 780 nm infrared laser were irradiated at 2 J/cm^2 at the wound edge and 2 cm around the wound. The output power for all laser wavelengths was set at 100mw. All patients showed rapid improvement in wound healing after 14 days of PDT and PBM treatment. This primary study evaluated the potential of photodynamic therapy (PDT) in combination with multi-wavelengths laser therapy for resistant chronic diabetic wounds. PDT and PBM combination has been shown to be successful and promising in improving clinical symptoms, reducing wound size, and preventing hospitalization, amputation, intensive care or major surgery. This treatment is easy to administer, inexpensive, and even can be done in a small clinics as well as hospitals.

KeyWords: Wound healing, Chronic Diabetic Ulcers, Wound Healing, Photodynamic therapy, Photobiomodulation.

Received 13.01.2023 Revised 16.03.2023 Accepted 21.04.2023

How to cite this article:
Michael W, Aref G, Yasaman Z M, Hans M W, Seyed M H K, ,Fatemeh Z M, Seyedeh P H K, Amirreza A, Seyed A G, Nahid R, Kamran M. Combination of Photodynamic Therapy and Multi-Wavelengths Photobiomodulation in Chronic Wound Healing Disorders: Case series. Adv. Biores. Vol 14 [3] May 2023. 110-118

INTRODUCTION

Photodynamic therapy (PDT) is one of the most interesting and promising approaches for treating various types of cancer and infectious diseases. PDT has a long history and is already approved for several superficial cancers of the esophagus, bronchial system, stomach, biliary tract, colon, and bladder.Unlike chemotherapy and radiotherapy, PDT treatment usually does not have serious side effects[1-3].

The principle is the stimulation of light-sensitive drugs (photosensitizers) that are applied to the skin as creams, applied orally, or injected into the bloodstream. In cancer treatment, photosensitizers are taken up by the abnormal and unhealthy tissues like tumor tissue by endocytosis and irradiated with specific wavelengths of light corresponding to the absorption spectrum of the photosensitizer. This photo activation process induces various chemical processes such as the generation of radical oxygen species that ultimately lead to the destruction of tumor tissue or microorganisms and viruses[2, 4].PDT is coming of an opportunity for microbial infections, a hassle that is currently irritated with the aid of using the more and more tremendous antibiotic-resistant microbial strains[5].

In particular, using violet or blue light-emitting up photosensitizers as photodynamic antimicrobial retailers is characterized with the aid of using diverse favorable features, including: (a) the large spectrum of antimicrobial movement, which leads the photosensitized inactivation of bacteria, fungi, mycoplasma, and parasites with the aid of using the use of one phototherapeutic protocol and slight irradiation conditions; (b) show no considerable toxicity withinside the darkish at photochemically lively doses; (c) microbial mobileular dying is generally a outcome of membrane photodamage via a usually multi-target process, which minimizes the hazard of each the onset of mutagenic techniques and the choice of photoresist ant cells; (d) such photosensitizers act with basically same performance towards each wild and antibiotic-resistant strains, while no choice of photoresist ant microbial pathogens has been observed; (e) an aggregate among antibiotic-primarily based totally and photodynamic remedy is possible[6-8]. At present, antimicrobial PDT seems to be mainly handy for the remedy of localized infections, together with oral candidiasis, periodontitis or persistent wounds[8].

Most photosensitizers used in therapeutic applications are derived from natural products, especially porphyrins such as chlorophyll, and are therefore called hematoporphyrins or chlorines. Additionally, other natural substances such as curcumin, hypericin, and riboflavin are available. They are effective photosensitizers and can also be stimulated according to their light absorption spectrum. We already knew what we could do. Antimicrobial photodynamic therapy (also called aPDT) is a treatment option based on the combination of photosensitizers that selectively localize to target tissues and the application of appropriate wavelengths of light to activate the photosensitizers that resulting in photo-damage and cell death[9, 10].

Exposure of the photosensitizer to light of a specific wavelength causes light absorption by the photosensitizer, which pushes the photosensitizer from the short-lived (nanosecond) state to the singlet-excited state. The singlet state photosensitizer can then undergo an electronic transition to the much longer-lived (microsecond) triplet state[2, 11].

Longer lifetimes allow triplet photosensitizers to react with ambient oxygen (ground state) via one of two different photochemical pathways, called type 1 and type 2 photochemical pathways. Type 1 involves electron transfer to generate superoxide radicals and then hydroxyl radicals, and type 2 involves energy transfer to generate singlet oxygen in the excited state. Superoxide, hydroxylradicals, and excited singlet oxygen damage nearly all types of biomolecules (proteins, lipids, and nucleic acids) and can kill cells by causing irreparable oxidative damage are highly reactive oxygen species (ROS). These processes are called photodamage. Typical examples of such damage are inhibition of protein synthesis and molecular alteration of DNA strands by DNA-protein cross-links or strand breaks. These processes alter the transcription of the genome during replication (mutagenic effects), ultimately leading to microbial death. Killing of microbial cells by PDT is rapid, lasting only seconds (whereas antibiotics and other drugs may take hours or days to take effect)[3, 5, 8].Light stimulation puts the photosensitizer into an excited state (PS*). Reaction with oxygen leads to reactive oxygen species (ROS) that can damage viral proteins, lipids, and nucleic acids[3, 5].

Riboflavin, also known as vitamin B2, is a vitamin found in foods and commonly used as a dietary supplement used as a supplement to treat all diseases related to vitamin B2 deficiency.It can be given orally or by injection. It is generally safe and well tolerated, even during pregnancy. Riboflavin is required by the body for cellular respiration. In humans, riboflavin is less soluble in water than other B vitamins and absorption efficiency decreases with increasing doses, so there is no evidence of riboflavin toxicity from overdose[1, 12].

Vitamin B2, along with other vitamin B complexes, is important for metabolism. It is a cofactor for enzymes involved in the metabolism and breakdown of proteins, fats, carbohydrates and purines. In addition, vitamin B2 is required for energy production. Vitamin B2 is also involved in the metabolism of other B vitamins, the production of hormones in the adrenal cortex, and the body's antioxidant defense system as a cofactor for the enzyme glutathione reductase. Therefore, vitamin B2 is very important in all stages of life. It is necessary for heart, brain, cognitive function, bones, joints, muscles, eyes, skin, immune system, liver, and more[1, 2, 13].

Riboflavin can be applied transdermal, orally, or intravenously. Due to the limited extent of absorption in the small intestine, intravenous administration of riboflavin can also be considered completely harmless. In chronic wounds, resorption occurs in specialized areas of open tissue. Topical application is very safe because drug absorption is limited by how long the drug is in contact with this area.
Riboflavin is widely marketed as a dietary supplement, with dosages available in formulations ranging from 100 to 400 mg. [14], which has much better water solubility. A safe dose of riboflavin-5-phosphate 100mg was recommended and used in this study for local effects.
The potential role of riboflavin in microbial photo-inactivation has been known for over half a century. Being a naturally occurring compound in the human body, riboflavin is easy to use for medical applications. From the outset, the application of riboflavin to photodynamic inactivation of viruses has attracted the most interest. It remains the most researched area, especially in decontamination of blood products. Due to our extensive knowledge of this naturally occurring compound, riboflavin has been certified as GRAS (generally regarded as safe) by the US FDA. It binds to the nucleobases of viral RNA and specifically oxidizes the guanine bases of nucleic acids through a single electron transfer reaction under short-wavelength light such as blue, violet, and ultraviolet. Subsequent reactions produce ½ O2, hydrogen peroxide, and hydroxyl radicals. This leads to irreversible single-strand breaks in the nucleic acid with damage to the pathogen[15, 16].Riboflavin is the active photosensitizer of the MIRASOL Pathogen Reduction Technology System (Terumo BCT, Lakewood, CO, USA) used for the treatment of platelet and plasma products. It is also used to reduce pathogens in whole blood. Other substances such as chlorine, hematoporphyrins, porphyrin, curcumin, methylene blue, and chlorophin also act as photosensitizers. We chose riboflavin because it is cheap, non-toxic, and easily absorbed. Another great advantage is that it works with blue and violet light. Both wavelengths, especially violet, are effective against viruses and microorganisms and meet all safety requirements[15-17].
Many studies support the antibacterial, antifungal, antiviral, and anti-parasitic effects of PDT, or short but safe wavelengths of light such as blue and violet, used to stimulate riboflavin in in vitro studies.
The emitted light is produced in blue wavelengths and is very effective. Violet and blue wavelengths are safe and effective in antibacterial effects[18-20]. These safety concerns led us to develop a new safe protocol based on a photodynamic mechanism using riboflavin as a photosensitizer in combination with violet, blue light stimuli. By using a photodynamic process, higher wavelengths and lower light energies are required to cause bacterial inactivation. This approach is considered safe for in vivo or in vitro applications as no side effects are expected. According to the absorption spectrum of riboflavin-5-phosphate, the absorption and excitation maxima are found at 375 nm and 447 nm[2, 21]. Red blood cells transport oxygen and nitric oxide (NO) in complexes. When red blood cells are exposed to blue light, free nitric oxide is released and blood vessels dilate rapidly. This effect can be seen as an additional benefit for thrombosis and embolic prevention. Therefore, in the presented study, several light-emitting diodes were equipped with a combination of blue wavelengths of 375 nm and 447 nm for maximum safety and therapeutic efficacy. We also used red wavelengths because riboflavin has a peak in red light as well[1, 2].
On the other side, there are many studies that prove the benefit of laser therapy and Photobiomodulation on wound healing and diabetic ulcers. This study hypothesizes that combination of PDT and PBM are effective on resistance wound and ulcers.

MATERIAL AND METHODS

Volunteers

Subject1: The patient's name is Mrs. Z S and he was born on November 20, 1972 in Behbahan, Iran. She suffered a diabetic foot injury in June 1919 and did not know she was diabetic. Her FBS was 400-600 mg/dl and she was injected with insulin but her FBS was still 200-300 mg/dl. She was admitted to Namazi Hospital, Shiraz Medical College on 7 June 1919, where she had been unsuccessfully treated for many months. Ultimately, surgeons at Namazi Hospital in Shiraz decided to amputate her left leg. She refused her amputation and accepted her amputation at a private diabetic and diabetic wound healing clinic in Tehran on 27 July 1919 and her PDT was started.
Subject 2: Mr. A.F, 52, lives in Amol. He has had insulin-dependent diabetes since 2009 and had a diabetic foot ulcer on his right foot in May 2019. Treatment with antibiotics showed no improvement. After an MRI of the patient's right bone osteomyelitis and blood work, an amputation of the ankle is performed. The patient was not amputated and was referred to my private clinic for diabetes and diabetic wound healing in Tehran and was immediately treated with diet and special bandages. He is resistant to diabetic foot ulcers. Then the medical board decided to amputate his leg and filed for PDT.
Subject 3:A female 47-year-old Tehran patient with diabetes since 2015, taking the drugs metformin and glibenglamide. She has been on insulin injections since 2018 because she couldn't control her blood sugar

and had diabetic foot ulcers since late 2018. She went to Chamran Hospital in Tehran for two months for the first checkup and bondage and antibiotics. After administering the substance, the patient's leg wound and osteomyelitis did not improve for two months, and the patient's blood sugar level was not controlled. Due to the patient's foot infection and uncontrolled osteomyelitis, as well as the patient's uncontrolled blood sugar, amputation of her knee was ordered by an orthopedic surgeon at Chamran Hospital in Tehran. The patient refused amputation and was referred to a private clinic for further treatment. An orthopedic surgeon at a private hospital in Tehran ordered an amputation of his knee and refused a blood transfusion because of anemia because his foot ulcer was so severe that he was in danger of dying the same day. The patient refrained from amputation and was referred to my private clinic for diabetes and diabetic wound healing in Tehran and was immediately treated with a diabetic diet and special bandages.

Subject 4: The patient's name is Mr. S.M.S., age 37, living in Arak, Iran. He has had insulin-dependent diabetes mellitus since 1993 and a diabetic ulcer on his left leg since March 2019. He made an emergency visit to Valiasr Hospital, Arak, Iran, where he received a two-month pre-examination and antibiotics and after treatment of the ulcer there was no improvement. Afterwards, he was admitted to the Valiasr Special Hospital in Arak. After his one-week stay at the Valiasr Special Hospital in Arak, his leg deteriorated and after consulting with a surgeon, the patient was transferred to Sina Special Hospital. He underwent three foot surgeries under anesthesia. They stopped treatment and discharged the patient without healing. The patient is then transferred to Tehran and admitted to Tehran Special Hospital Mer. After the surgeons visited the patient and took an MRI and blood tests for osteomyelitis in the patient's left bone, they decided to amputate his left leg. The patient did not undergo amputation and was referred to PDT.

Subject 5:A female 57-year-old Tehran patient with diabetes since 2017, on insulin since late 2018, unable to control blood sugar and suffering from diabetic foot ulcers. She was hospitalized in three different hospitals in Iran, she received many drugs and antibacterial therapy, but her wounds got better and the final hospital team decided to amputate her leg, leaving her refused and was referred to PDT.

Procedure

Riboflavin-5-phosphate (100mg capsules) was provided by UltraBotanica (Oklahoma, USA). All analysis certificates and product specifications were provided by UltraBotanica.The PDT equipment was developed and provided by W Medical Systems GmbH (Germany)with three different wavelengths, and the laser therapy device was provide RJ LASER, Germany (Polylaser derma).

We open Riboflavin-5-Phosphate (100mg) capsule, dissolve in a glass with 20 ml of sterile water and spray onto the wound area for topical application. Spray onto the wound areas.

We applied blue laser (447nm) and violet laser (375 nm) on a wound area, and we applied red laser (630nm) and infrared laser (780nm), 2 cm far from the wound edges, around the wound.

The output power for all wavelengths was set to 100mW. The energy density was set at 10 joules per square centimeter for the wound area, and was set at 2 joules per square centimeter around the wound area. The wound surface is irradiated with violet and blue wavelength laser shower, and the red and infrared laser are irradiated locally around the wound.

RESULTS

Figure 1. Subject 1, in the top left, the wound is before starting PDT, and laser therapy. In the top right, the results are after the first week. In the bottom left, the results are after the second week, and in the bottom right, the results are after the fifthweek.

Figure 2. Subject1, on the left, the wound is after the first week of PDT combination with laser therapy. In the middle center, the results are after the second week. On the right, the results are after the fifth week.

Figure 3. Subject2, on the left, the wound is before starting the PDT and PBM. In the middle center, the results are after the second week. On the right, the results are after the fourth week.

Figure 4. Subject3, on the left, the wound is before starting the PDT and PBM. In the middle center, the results are after the second week. On the right, the results are after the sixth week.Figure 5. Subject3, on the left, the wound is after the second week of PDT and PBM. In the middle center, the results are after the fourth week. On the right, the results are after the sixth week.

Figure 6. Subject3, on the left, the wound is before starting the PDT and PBM. On the top right, the results are after the second week. On the bottom right, the results are after the sixth week.

Figure 7.Subject 4, on the left, the wound is before starting PDT and PBM. On the right, the results are after the fourth week of PDT and multi wavelengths laser therapy.

Figure 8. Subject 5, on the left, the wound is before starting PDT and PBM. On the right, the results are after the sixth week of PDT and multi wavelengths laser therapy.

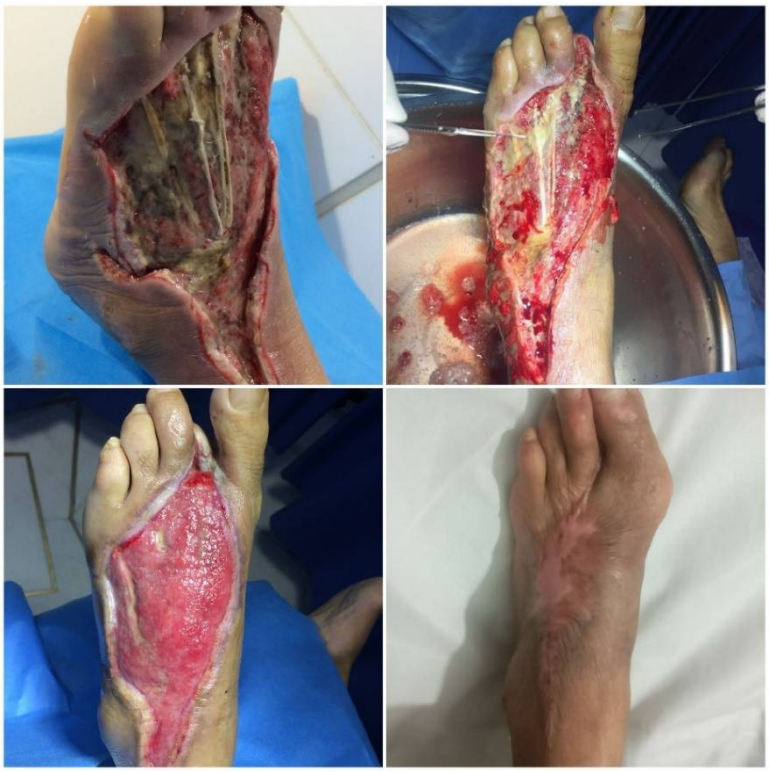

Figure 1. Subject 1, in the top left, the wound is before starting PDT, and laser therapy. In the top right, the results are after the first week. In the bottom left, the results are after the second week, and in the bottom right, the results are after the fifthweek.

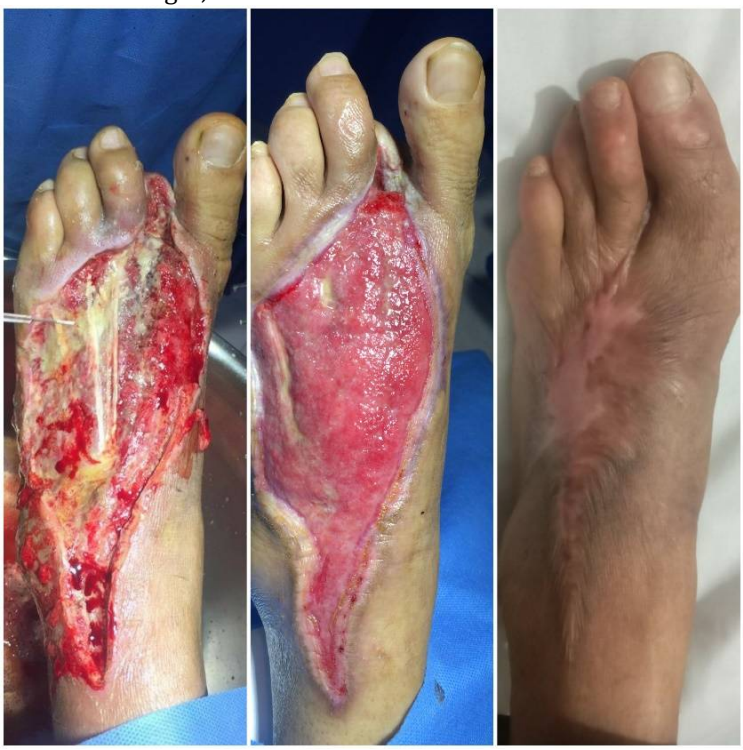

Figure 2. Subject1, on the left, the wound is after the first week of PDT combination with laser therapy. In the middle center, the results are after the second week. On the right, the results are after the fifth week.

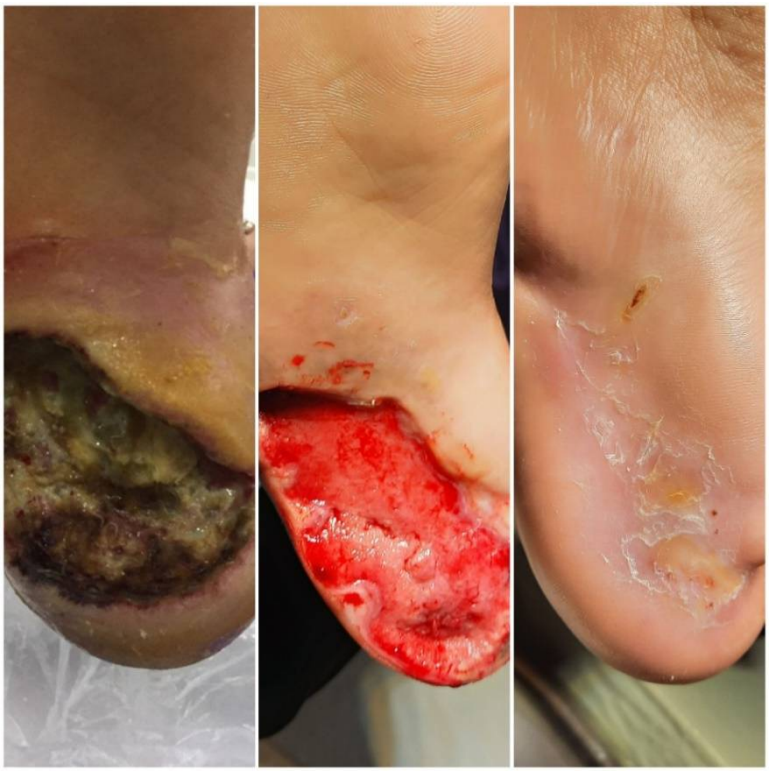

Figure 3. Subject2, on the left, the wound is before starting the PDT and PBM. In the middle center, the results are after the second week. On the right, the results are after the fourth week.

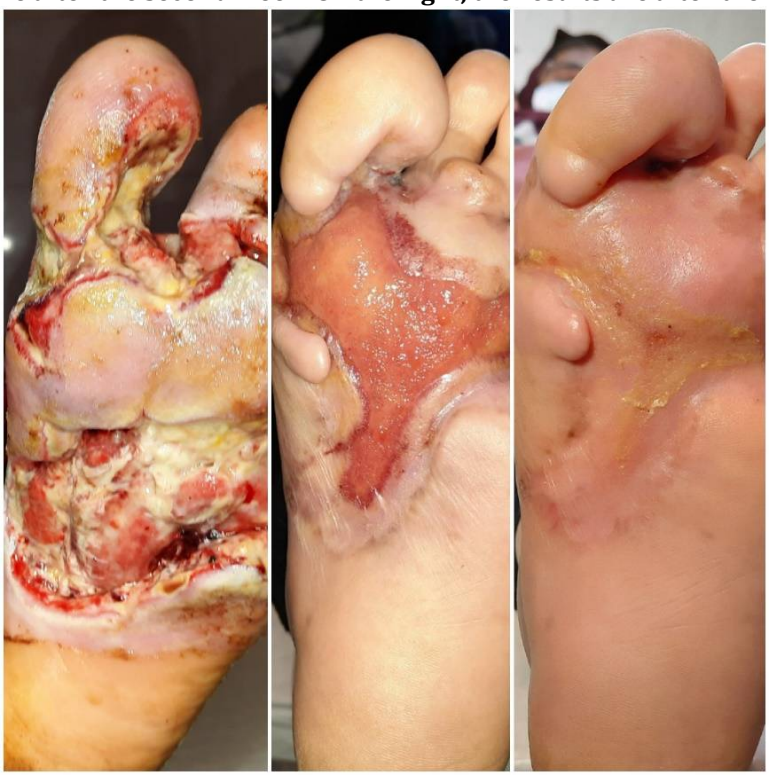

Figure 4. Subject3, on the left, the wound is before starting the PDT and PBM. In the middle center, the results are after the second week. On the right, the results are after the sixth week.

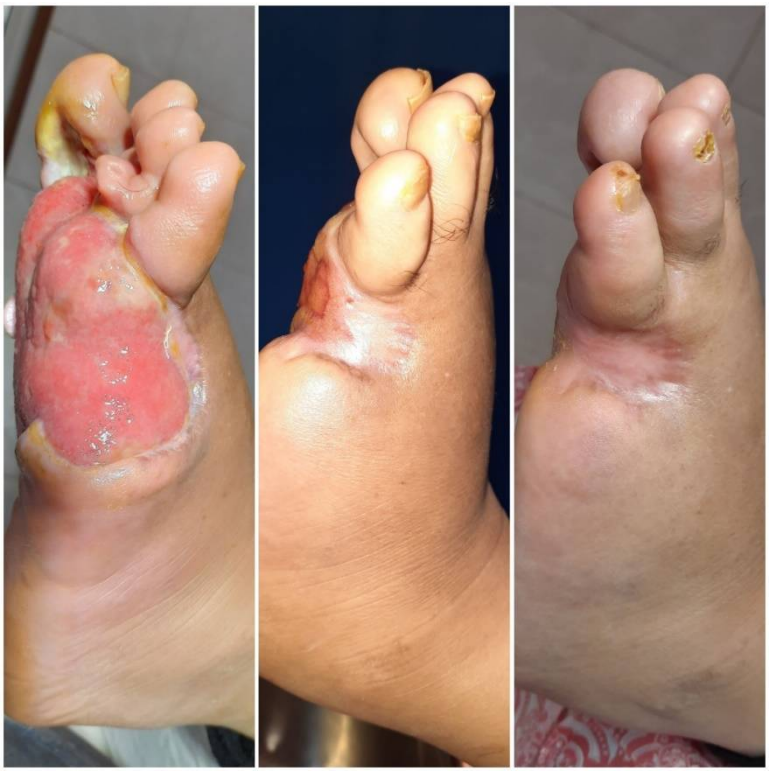

Figure 5. Subject3, on the left, the wound is after the second week of PDT and PBM. In the middle center, the results are after the fourth week. On the right, the results are after the sixth week.

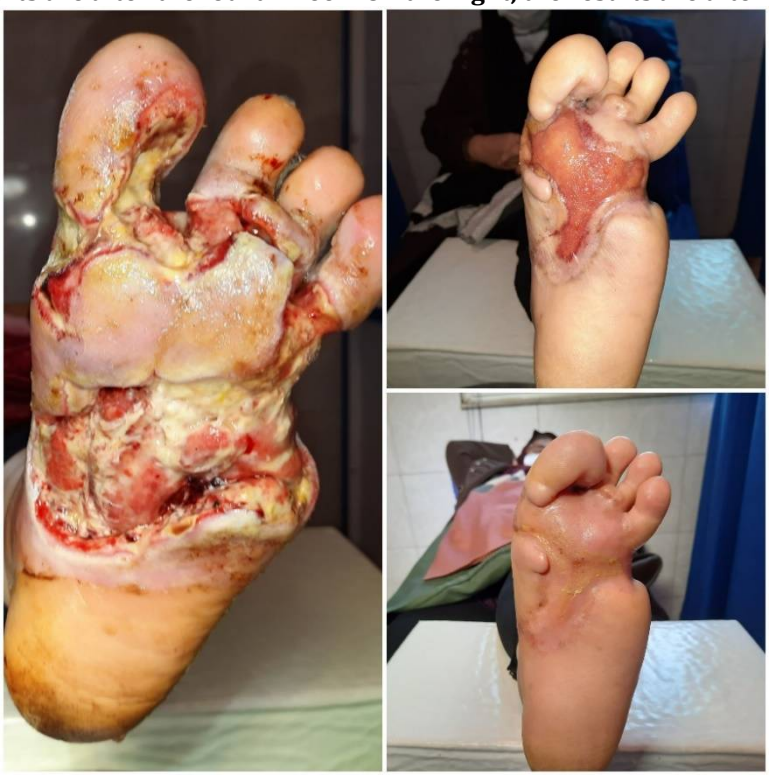

Figure 6. Subject3, on the left, the wound is before starting the PDT and PBM. On the top right, the results are after the second week. On the bottom right, the results are after the sixth week.

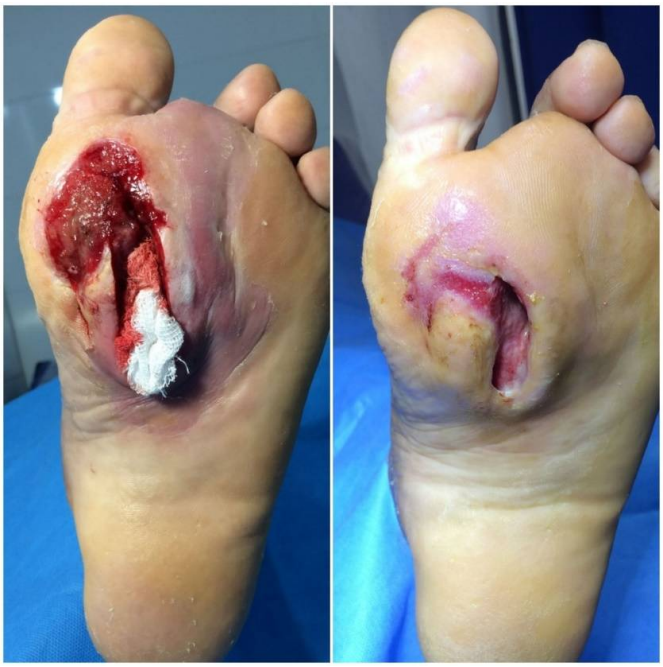

Figure 7.Subject 4, on the left, the wound is before starting PDT and PBM. On the right, the results are after the fourth week of PDT and multi wavelengths laser therapy.

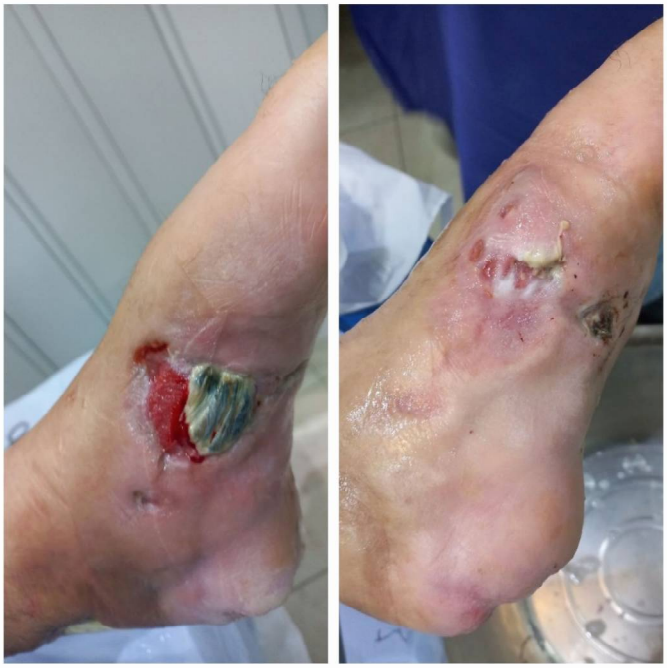

Figure 8. Subject 5, on the left, the wound is before starting PDT and PBM. On the right, the results are after the sixth week of PDT and multi wavelengths laser therapy.

DISCUSSION AND CONCLUSION

The efficacy of photodynamic therapy (PDT) for infected wounds has been demonstrated in several recent in vitro and in vivo experiments. Several patients with resistant wounds and infections were treated at the clinic and experienced immediate clinical improvement. Results show high efficacy in improving clinical symptoms, reducing bacterial load, and promoting healing in patients with diabetic ulcers[1, 2, 7, 9].

Conventional treatment did not show significant improvement in the feet and ulcers in diabetic patients, but essentially all five patients in the study experienced significant reductions in ulcer size and improvement in deep ulcers. In addition, we emphasize that PDT caused no side effects. This is consistent with all in vivo and in vitro and safety data for riboflavin-based photodynamic therapy. Another

advantage of PDT is that it is easy to use and inexpensive. This will not only reduce the burden on hospitals with intensive care units, but also prevent overspending by health insurers and damage to the global economy[1, 7, 10].

Conventional drug therapy has had very limited success in terminal wounds. However, our treatment approach cannot be applied to complementary medicine. Successful treatment of such terminal wounds requires a combination of Western pharmacotherapy and complementary medicine such as photodynamic therapy (PDT) for chronic wounds. The presented study provides evidence of successful treatment of chronic diabetic ulcers in end-stage diabetic patients with photodynamic therapy. An application protocol combining topical riboflavin with violet, blue and laser therapy using red and infrared light proved effective in improving the regeneration and healing process of diabetic ulcers. This therapeutic intervention is suitable for both early and late stages of wounding.

REFERENCES

1. Weber, H.M., et al., (2020). Successful Reduction of SARS-CoV-2 Viral Load by Photodynamic Therapy (PDT) Verified by QPCRÃ¢ Â? Â? A Novel Approach in Treating Patients in Early Infection Stages. Medical & Clinical Research. **5**(11): p. 311-325.
2. Weber, M., et al., (2021). Anti-viral photodynamic therapy in Covid-19 management: A novel approach in treating patients in early infection stages. Akupunktur & Aurikulomedizin, **47**: p. 29-34.
3. Dolmans, D.E., D. Fukumura, and R.K. Jain,(2003). Photodynamic therapy for cancer. Nature reviews cancer, **3**(5): p. 380-387.
4. Agostinis, P., et al., (2011). Photodynamic therapy of cancer: an update. CA: a cancer journal for clinicians, **61**(4): p. 250-281.
5. dos Santos, A.l.F., et al., (2019). Photodynamic therapy in cancer treatment-an update review. Journal of cancer metastasis and treatment, **5**: p. 25.
6. Huang, Z., (2005). A review of progress in clinical photodynamic therapy. Technology in cancer research & treatment, **4**(3): p. 283-293.
7. Macdonald, I.J. and T.J. Dougherty, Basic principles of photodynamic therapy. Journal of porphyrins and Phthalocyanines, 2001. **5**(02): p. 105-129.
8. Kharkwal, G.B., et al., Photodynamic therapy for infections: clinical applications. Lasers in surgery and medicine, 2011. **43**(7): p. 755-767.
9. Liu, Y., et al., Antibacterial photodynamic therapy: overview of a promising approach to fight antibiotic-resistant bacterial infections. Journal of Clinical and Translational Research, 2015. **1**(3): p. 140.
10. Marhold, F., et al., Does pigmentation, hemosiderin and blood effect visible 5-ALA fluorescence in cerebral melanoma metastasis? Photodiagnosis and Photodynamic Therapy, 2022. **39**: p. 102864.
11. Litscher, G. and M.H. Weber, 10th Anniversary of the International Society for Medical Laser Applications. Medical Acupuncture, 2022. **34**(4): p. 217-219.
12. Powers, H.J., Riboflavin (vitamin B-2) and health. The American journal of clinical nutrition, 2003. **77**(6): p. 1352-1360.
13. Rivlin, R., Riboflavin. 2012: Springer Science & Business Media.
14. MERRILL JR, A.H., (1979). Studies On The Formation Of Riboflavin 5'-Phosphate By Flavokinase And Its Utilization By Pyridoxamine (Pyridoxine) 5'-Phosphate Oxidase. Cornell University.
15. Goodrich, R.P., et al., (2006). The Mirasol™ PRT system for pathogen reduction of platelets and plasma: An overview of current status and future trends. Transfusion and apheresis science, **35**(1): p. 5-17.
16. Li, J., et al., (2005). Evaluation of platelet mitochondria integrity after treatment with Mirasol pathogen reduction technology. Transfusion, **45**(6): p. 920-926.
17. Cazenave, J.P., et al., (2010). A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology. Transfusion, **50**(11): p. 2362-2375.
18. Zupin, L., et al.,(2018). Antiviral properties of blue laser in an in vitro model of HSV-1 infection. Microbiology and Immunology, **62**(7): p. 477-479.
19. Rupel, K., et al., (2019). Blue laser light inhibits biofilm formation in vitro and in vivo by inducing oxidative stress. NPJ biofilms and microbiomes, **5**(1): p. 29.
20. de Sousa, N.T.A., et al., (2015). Blue laser inhibits bacterial growth of Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Photomedicine and laser surgery, **33**(5): p. 278-282.
21. Bhargava, S., et al., (2020). Effect of blue light and photosensitizers on Cutibacterium acnes on shoulder periprosthetic joint infection isolates. Journal of Bone and Joint Infection, **5**(4): p. 187-197.