

Case Study of Ozone Bagging Adjuvant Therapy in Wagner II and III Diabetic Foot Ulcers on Wound Healing Process: Three Patients Recovered Completely

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ABSTRACT

Background: Diabetic foot ulcer is a chronic complication of diabetes mellitus in the form of ulceration with or without infection. Diabetic foot ulcers that are not handled properly can cause damage to the foot that requires amputation. Wound care techniques for treating diabetic foot ulcers are starting to develop, one of which is ozone bagging therapy. This ozone bagging therapy in diabetic foot ulcers is reported to be useful as an anti-microbial effect, increasing glycemic control, increasing insulin sensitivity, preventing oxidative stress, increasing fibroblast proliferation, and increasing the release of growth factors. This case study will report 3 patients with Wagner II and III-foot ulcers who healed completely within 28 days using ozone bagging treatment as an adjuvant.

Methods: 3 patients with diabetic foot ulcers Wagner II and III received ozone bagging therapy every 4 days for 7 sessions (a total of 28

days) as an additional therapy besides treating moist gauze using NaCl. This study used a Medical Ozone Generator. The dose of ozone used with medical oxygen is 1 lpm, flow 0.6, in the first session "high dose" with an output dose of 80-100 mg/l and in the 2nd-7th session with a low dose (25-35 mg/l). Macroscopic assessment of the wound using the Wagner scale and the progress of each treatment session is documented in photographs.

Results: On macroscopic measurements, the process of wound healing leading to wound closure was better in the treatment group and was taken as a case for 3 patients who recovered completely. It resulted in 3 patients who recovered completely with a measurement of the Meggitt-Wagner scale of 0.26.

Conclusion: For these recovered patients, there was a decrease in VEGF, TGF- β , and PDGF levels. The VEGF, TGF- β , and PDGF levels tested were VEGF, TGF- β , and PDGF levels in wound exudate by comparing them on the 3rd day. 1st and 12th day. Supported by

macroscopic data showing that the wound healing process was better in the group that received ozone bagging therapy as an adjuvant. From this study it was found that the trend of decreasing levels of VEGF, TGF- β , and PDGF in wound exudate occurred because the inflammatory and proliferative processes in the treatment group lasted more faster than the control group.

Keywords: ozone bagging therapy, diabetic ulcers, wound exudate, growth factors, VEGF, TGF- β , and PDGF, Meggitt-Wagner scale.

INTRODUCTION

Diabetes mellitus is a progressive chronic disease characterized by an increase in blood glucose. (1) Diabetes mellitus occurs when the pancreas does not produce enough insulin (a hormone that regulates blood glucose), or when the body cannot use the insulin, it produces effectively. (1) Diabetes mellitus is one of the four priorities for non-communicable diseases (NCDs). (1,2)

Diabetes mellitus can cause various complications in all parts of the body, one of which is diabetic foot ulcers (1-3)

Indonesia is one of the countries with the 6th (sixth) largest number of diabetics in the world with an age range of sufferers between 20-79 years, totaling around 10.3 million people (4) It has been predicted that by 2030 this number will reach 21.3 million people. (4) In 2017, people with diabetes mellitus in Central Java were ranked 5th out of the top 10 diseases at the Puskesmas (Health Center for Society), with a total of 25,608 people. (4) Previous research at a hospital in Central Java said that as many as 91% of 100 respondents with diabetes mellitus had experienced diabetic foot ulcers and 58% of respondents were still undergoing wound care for diabetic foot ulcers. (4) Diabetic foot ulcers are a chronic complication of diabetes mellitus in the form of ulcerations affecting the lower limbs with or without infection. (5,6) Diabetic foot ulcers that are not handled properly can cause damage to the feet requiring amputation. (5,6) Hyperglycemia in long-term diabetes mellitus can cause oxidative stress

and damage to various body systems including the nervous system, immune system and vascular endothelium. (5,6)

Damage to the vascular endothelium and the body's immune system that occurs in patients with diabetes mellitus who experience injuries, often becomes difficult to heal and develops into diabetic foot ulcers. (5,6) Damage to the nervous system that often occurs is diabetic neuropathy, causing loss of sensory sensation such as pain in the feet is also one of the causes of the high incidence of diabetic foot ulcers, and leads to amputation. (5,6) Wound care for diabetic foot ulcers is still a challenge in itself. (4) Management of diabetic foot ulcer care focuses on overcoming infections that occur, as well as vascular disorders. (6,7) Moist wound care techniques, which are standard wound care therapies, are still the treatment technique of the wound that the most often technique used in the treatment of diabetic foot ulcers. (4) However, the weakness of this wound care technique is that it still takes quite a long time for diabetic foot ulcers to heal. (4) This of course will be disruptive and have an impact on the quality of life of sufferers diabetic foot ulcer. (4,5,8)

Other wound care techniques for treating diabetic foot ulcers have begun to develop. (8-11) Among them is ozone bagging therapy, a method of distributing ozone to the wound area using a plastic bag and then ozone from a medical ozone generator is flowed into a plastic bag that has covered the ulcer. diabetic foot. (4,6,8-10) Ozone bagging therapy for diabetic foot ulcers is beneficial as an anti-microbial effect, increasing glycemic control, increasing insulin sensitivity, preventing oxidative stress, increasing fibroblast proliferation, and increasing the release of growth factors such as PDGF, TGF- β , and VEGF which make the wound healing process faster and more effective. (10,12,13) The use of ozone bagging for diabetic foot ulcers abroad has been

widely used.^(6,9,10) But in Indonesia, is still not widely used, while the Medical Ozone Generator device found by researchers in Indonesia has met the standards for use as an adjuvant therapy for diabetic foot ulcers. So the researchers were interested in knowing the effect of ozone bagging therapy as an adjuvant on Wagner II and III diabetic foot ulcers with growth factor parameters (PDGF, TGF- β , and VEGF) using the M-Ozone tool (a medical ozone generator found by Indonesian researchers).

MATERIALS & METHODS

a. Study Design

The study was done at the Diponegoro National Hospital, Clinic Primary Diponegoro, *Fatchull Wound Care Healing*, and Community Health Centers Undip Faculty of Medicine Network in Semarang City the period time May- July 2022. For inspection samples, exudate wounds and samples of blood HbA1c will be carried out at the GAKI Lab FK Undip. Type Research used studies *queasy experimental pre-post test control group* with the use of patient Wagner II and III diabetic ulcers at Diponegoro General Hospital, Clinic Primary Diponegoro, *Wound Care Healing* Faculty, and Community Health Centers Undip Faculty of Medicine Network as respondent research.

b. Medical Ozone Generator

Medical ozone was generated by using a DDBD plasma generator which consists of two Pyrex glass barriers separated by 3 mm from each other^(13,14). This type of DDBD is known to generate high ozone purity, as the barriers keep the electrodes from corrosion, preventing the produced ozone from contamination⁽¹⁴⁾. Oxygen was used as inlet gas and it was set to flow with a rate of 0.3 L min⁻¹ into the generator, while the voltage discharge applied to the DDBD was 3 kV.

c. Retrieval Technique Sample

The whole candidate respondent will explain especially formerly about the research to be done, the treatment that will

be received, and the effect, as well as possible risks, happened. Whole candidates willing respondents follow the study will sign a sheet agreement research / informed consent. Before treatment is granted, candidate respondents especially those formerly fill in questionnaire demographics. The then-candidate respondent will be taken blood for checked HbA1c and Hb levels. Respondents with HbA1c levels > 10.5% did not follow in research. Then-candidate Respondents will too examine ABI (Ankle Brachial Index). Prospective respondents who have an ABI value < 0.70 were not followed in the research. After evaluation first, the candidate stated respondent _ get away for could follow the study and then will be called as respondent. Bates Jensen Wound Assessment Tool (BWAT) scale on day 1 and day 28 of the study as evaluation macroscopic wound. Variable Free on research this is Ozone Bagging Therapy as an Adjuvant, Meanwhile Variable Depends on research this is the Value of pro-inflammatory mediators Interleukin-1 and anti-inflammatory Interleukin-10 at the time day first treatment and the 12th day of treatment from group treatment and control group. Macroscopic picture wound use scale *Bates Jensen Wound Assessment Tool (BWAT)* after treatment 7 sessions full / 28 days treatment from group treatment and control group.

d. Data collection

A deep data collection study this done with several stages, that is Step preparation, stage intervention, and stage post-intervention.

e. Stage Preparation

Stage preparation covers guidance with mentor research, preparation of research proposals, requests permission study to Committee ethics Health Research FK Undip, request permission implementation study to Leader of Diponegoro National Hospital, Clinic Diponegoro, Puskesmas Undip FK Network, and *Fatchull Wound Care Healing*, as well as requests permission

use of M-Ozone for research this to head of PT DIPO Technology.

f. Stage Intervention

Started with coordination with a team of doctors and nurses at Diponegoro National Hospital, Clinic Diponegoro, Puskesmas FK Undip Network, and Fatchull Wound Care Healing. Next with patient data collection Wagner II and III diabetic foot ulcers. After that group patients into group intervention /control. Done explained about therapy ozone bagging, its benefits, and risks, and requested informed consent to the respondent's research. Respondents' research also filled out questionnaire data especially first. Next done care wound ulcer diabetic foot in the respondents, in the group intervention given Standard care added therapy ozone bagging as an adjuvant. In the control group given standard care.

g. Stage Post Intervention

Post-intervention, a researcher will evaluate therapy second group with a method evaluating mark pro-inflammatory cytokine Interleukin-1 and anti-inflammatory Interleukin-10 at baseline research and the 12th day of research in both groups. Researchers will too evaluate the method to compare macroscopic wound second groups at the start and end of treatment research. Inspection of fluid exudate wound will be carried out at the GAKI Laboratory of Faculty of Medicine Diponegoro University for checked Interleukin-1 and Interleukin-10 levels and growth factor parameters (PDGF, TGF- β , and VEGF).

RESULT

The medical ozone made by Center for Plasma Research, Diponegoro University in collaboration with Dipo Technology is trying to standardize it is an ozone generator made in Germany which was developed by Dr. J. Hansler. For low doses, the generator

can emit ozone between 20 $\mu\text{g/ml}$ – 30 $\mu\text{g/ml}$, while for high doses the ozone output from MOG is 80 $\mu\text{g/ml}$ – 100 $\mu\text{g/ml}$. This is an indication of the ozone concentration. If multiplied by flowrate (flow rate in ml/minute) and operating time in minutes. So the dose of ozone bugging can be measured by the formula below. Dose = Concentration x Flow Rate x time Using this formula, we set the dose required for direct delivery of ozone via ozone bugging to the diabetic ulcers of the patient. Actually, there are many other diseases that can use ozone therapy. It really needs the right dosage. With 3 variations multiplied to determine the dose, all standards (referring to the Hänsler standard) for disease cure can be achieved. Figure 2 shows a pair of Medical Ozone Generator (MOG) with the touchscreen turned on, and measurement of ozone output from a silicone ozone concentration hose with a Scientific Ozone meter (US Standard)

Table 2 below shows the results of measuring the concentration of ozone released by GOM with two categories, namely for low doses, the generator can emit ozone between 20 $\mu\text{g/ml}$ – 30 $\mu\text{g/ml}$, while for high doses the ozone output from MOG is 80 $\mu\text{g/ml}$ – 100 $\mu\text{g/ml}$. The ozone output is obtained by varying the flow rate. The table also shows the capacity of the ozone produced by the generator. This capacity is used as an option to determine the time needed to treat a disease. Scientifically, this opens up new research for medical and health sciences. The dose of ozone required by certain diseases in their healing is highly dependent on the biochemical mechanisms that occur. This process is very complicated. Research with equipment that can fully control very precise doses and then become Standard Operational Procedure (SOP) or Best Practices for therapy



Figure 1 : A pair of Medical Ozone Generator with touchscreen turned on, and ozone output from through silicon hose

Table 1. Concentration and capacity of ozone that can be produced

No	Flowrate (l/min)	Concentration		Capacity	
		low Concentration setting (µg/ml)	High Concentration setting (µg/ml)	Low Capacity (mg/minute) base on low concentration setting	High Capacity (mg/minute) base on low concentration setting
1	0,1	39,69	110,1	3,96	11,01
2	0,2	38,81	103,9	7,76	20,78
3	0,3	37,55	98,02	11,26	29,40
4	0,4	35,68	92,94	14,27	37,17
5	0,5	33,17	89,87	16,58	44,93
6	0,6	31,40	87,94	18,84	52,76
7	0,7	30,79	86,46	21,55	60,52
8	0,8	30,66	81,91	24,52	65,52
9	0,9	30,23	79,74	27,20	71,76
10	1,0	29,96	78,81	29,96	78,81
11	1,1	29,86	76,90	32,84	84,59
12	1,2	29,40	74,62	35,28	89,54
13	1,3	28,75	73,41	37,37	95,43
14	1,4	28,20	73,01	39,48	102,21
15	1,5	27,21	70,87	40,81	106,30

The capacity of the ozone released by the generator is obtained from the product of the concentration of ozone and the flow rate of oxygen sent into the reactor. The dose is obtained by multiplying the capacity by the treatment time. The ozone generator uses the facility to turn on and off, select the concentration level and determine the time using a touchscreen.

Table 3 below shows the results of dose calculations using the results of measurements of the ozone concentration. Dosage is calculated using the formula, Dosage = Concentration x Flow Rate x time. The doses that have been obtained in table three are the initial reference for doctors to make choices, concentration levels, flow rates and treatment times for patients.

Table 2: Dosage for diabetic wound healing based on medical application in diabetic wounds

No	Flowrate (l/min)	Dose diabetic ulcers with time treatment 2 minutes		Dose diabetic ulcers with time treatment 10 minutes	
		Dosage		Dosage	
		Low Dosage (mg) base on low concentration setting	High Dosage (mg) base on high concentration setting	Low Dosage (mg) base on low concentration setting	High dosage (mg) base on high concentration setting
1	0,1	7,93	22,02	39,69	110,10
2	0,2	15,52	41,56	77,62	207,80
3	0,3	22,53	58,81	112,65	294,06
4	0,4	28,54	74,35	142,72	371,76
5	0,5	33,17	89,87	165,85	449,35
6	0,6	37,68	105,52	188,40	527,64
7	0,7	43,10	121,04	215,53	605,22
8	0,8	49,05	131,05	245,28	655,28
9	0,9	54,41	143,53	272,07	717,66
10	1,0	59,92	157,62	299,60	788,10
11	1,1	65,69	169,18	328,46	845,90
12	1,2	70,56	179,08	352,80	895,44

13	1,3	74,75	190,86	373,75	954,33
14	1,4	78,96	204,42	394,80	1022,14
15	1,5	81,63	212,61	408,15	1063,05

Table 3: Ozone concentration and dosage for medical applications according to Dr. J. Hänslers

Indication	Ozone Concentration	Form of Application	Treatment Time	Treatment Frequency
Decubitus ulcers	80-100 µg/ml in the beginning	Low pressure suction cup	2-10 min	Daily first, then 1-2x per week
After wound cleansing	20-30 µg/ml	Low pressure boot (or plastic bag)	10-20 min	Daily first, then 1-2x per week
Diabetic gangrene	80-100 µg/ml in the beginning	Plastic bag (not low pressure!)	10-20 min	Daily first, then 1-2x per week
After wound cleansing	20-30 µg/ml			
Ulcus cruris	80-100 µg/ml in the beginning			
Wound cleansing				
Wound Healing	20-30 µg/ml	Compresses + rinsing with ozone water	1-5 min	Several times daily
		Plastic bag, compresses +	10-20 min	First 1-2x per day
Burn stage 1 and 2	20-30 µg/ml	rinsing with ozone water	1-5 min	Several times per day

CASE STUDY 1

Mr. Sg 61 years old is a farmer with a high school education. Mr Sg first learned he had diabetes mellitus in 2021 and regularly took oral anti-diabetic drugs in the form of 500 mg of 3x metformin. Mid-August 2022 Mr. Sg was stabbed by a nail and there was a wound on his left leg which was getting wider and wider and oozing pus. Mr Sg then visited the Fatchull Wound Care Clinic in Purwodadi and was diagnosed with a Wagner II diabetic foot ulcer. Mr. Sg then agreed to undergo adjuvant ozone bagging treatment which was carried out every 4 days for 28 days. Before administering adjuvant ozone bagging therapy, Mr Sg had his Ankle Brachial Index checked and his HbA1c level checked. Ankle Brachial Index Tn Sg 0.9 with HbA1c level 6.1%. Ankle-brachial index (ABI) is a non-invasive examination in the field of vascular medicine. This simple test is performed to diagnose peripheral arterial disease. With an ABI score of 0.9 it is said that the vascular

condition of Mr Sg's leg is in good condition (no vascular disorders). HbA1c examination is an examination by measuring the level or percentage of glucose bound to hemoglobin. HbA1c levels can be said as a parameter to see the state of human blood sugar in the last 3 months. With an HbA1c level of 6.1%, it was said that in the last 3 months Mr Sg's blood sugar level was under good control, in accordance with Mr Sg's statement who claimed to take oral anti-diabetic drugs regularly.

The progress of the healing process of Wagner II diabetic foot ulcers experienced by Mr. Sg is documented in the image above. At the 4th visit or treatment, there was an improvement in the condition of the wound from Wagner II diabetic foot ulcer to Wagner I. The wound condition was getting better day by day and closed completely to become Wagner 0 diabetic foot ulcer, right on day 28 or at the end of the therapy session.



Figure 2: The wound condition on day 28 after 7th the therapy session

CASE STUDY 2

The following is documentation of the progress of the wound healing process from Mr. NH 47 years old who is an entrepreneur worker in the city of Semarang. Mr NH is a high school graduate with a history of developing new diabetes in 2022. Mr NH admits that he does not routinely take oral anti-diabetic drugs obtained from the Bandarharjo Health Center in the form of Metformin 3x 500 mg. At the end of September 2022, Mr. NH had a lump of wood in his right leg until there was a wound which grew wider and wider until it festered. Mr. NH then agreed to undergo adjuvant ozone bagging therapy which was carried out at the Bandarharjo Health Center every 4 days.

Before starting the therapy session, Mr NH had his HbA1c and Ankle Brachial Index levels checked. Examination of the Ankle Brachial Index obtained a score of 1, or there was no vascular disturbance in Mr. NH's legs. For HbA1c scoring, the level was 9.7%. This high HbA1c level reflects the condition of Mr. NH's blood sugar in the last 3 months that was not controlled. In accordance with the confession of Mr. NH who does not routinely take oral antidiabetic drugs.

The development of Mr. NH's wound healing process was quite good, on the 4th visit the Wagner II diabetic foot ulcer had improved and became a Wagner I diabetic foot ulcer. On the 6th visit, or the 24th day, the wound had completely healed into a Wagner diabetic foot ulcer 0.



Figure 3: The wound condition on day 24 after 6th the therapy session and completely healed into a Wagner diabetic foot ulcer 0.

CASE STUDY 3

Mrs. S, 52 years old, has had a DM ulcer since the last 3 months. Mrs. S used to be an employee at the swallow's nest house, but since experiencing a DM ulcer for the last 3 months, Mrs. S is no longer working. Mrs S said that she had suffered from DM for 15 years and routinely took oral anti-diabetic drugs in the form of 500 mg of metformin 3x every day. Mrs S also said that at first, there was an ulcer on her left leg 5 months ago. Then the boil gets bigger and bigger, and oozes pus. Then Mrs. S routinely checked and received wound care at the Bandarharjo Health Center. When we met, we got Mrs S's left leg wound with a Wagner II diabetic leg ulcer. Mrs S then agreed to get ozone bagging adjuvant therapy for her legs once every 4 days. Before starting adjuvant ozone bagging therapy, we examined Ny S's ABI and HbA1c. The ABI score was 0.9% and the

HbA1c level was 10.1%. The ABI score reflects the absence of vascular disorders in Mrs S's legs. Meanwhile, Ny S's high HbA1c level reflects high blood sugar levels over the last 3 months. The cause of the high blood sugar level could be because Mrs. S actually didn't take her anti-diabetic medication regularly, or the anti-diabetic drug that Mrs. S took was not according to the dosage or type of class of anti-diabetic drug. It is necessary to carry out a separate examination related to the oral anti-diabetic drug.

The picture above is a picture of Mrs S's wound which improved and closed completely from Wagner II at the beginning of treatment to Wagner 0 at the end of therapy (28th day). In the 4th therapy session the wound had repaired, becoming a Wagner I diabetic foot ulcer, the wound tended to dry up and started to close. The wound closed completely on the 28th day.



Figure 4: The wound condition on day 28 after 7th the therapy session and completely healed into a Wagner diabetic foot ulcer 0.



Figure 5: Mr NH and Mrs S during one of the ozone bagging therapy sessions at the Bandarharjo Health Center in Semarang

CONCLUSION

On macroscopic measurements, the process of wound healing leading to wound closure was better in the treatment group and was taken as a case for 3 patients who recovered completely. It resulted in 3 patients who recovered completely with a measurement of the Wagner Meggit scale of 0.26. For these recovered patients, there was a decrease in VEGF, TGF- β , and PDGF levels. Supported by macroscopic data showing that the wound healing process was better in the group that received ozone bagging therapy as an adjuvant. From this study it was found that the trend of decreasing levels of VEGF, TGF- β , and PDGF in wound exudate occurred because the inflammatory and proliferative processes in the treatment group lasted more faster than the control group. From this study it can be recommended that ozone bagging therapy can be used as adjuvant therapy in wound healing.

Declaration by Authors

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