

Original Research Article

Improvement the Healing Rate of Diabetic Foot Wound Using Low Pressure Oxygen (TPOT), Negative Pressure (VAC) and alternating combined VAC and TPOT

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Abstract: Study the effect of low pressure, oxygen therapy (TPOT), and negative pressure wound therapy and the combination of both as three adjuvant therapies were analyzed to assess the rate of healing of diabetic foot wound. Our study was done on 20 patients with difference diabetic foot ulcer sizes. Our results revealed a higher rate of healing when using alternating combined VAC and TPOT in either good perfuse limb or in a poor perfuse limb as indicated with ABPI, in addition; the total duration for complete healing showed high significant shortening when using this combined therapy. Success in wound care may be improved by a personalized wound care therapy. The key lies in our ability to raise the normoxia set point of the wound by transient TPOT that allows the VAC angiogenesis hypoxic response to act with adequate wound oxygenation that maintain the viability of the deep pockets and allows tissue repair.

Keywords: low pressure oxygen (TPOT), negative pressure (VAC), MIX, diabetic wound.

INTRODUCTION:

Patients with diabetes often develop foot ulcers due to atherosclerosis that impedes blood flow to the extremities and peripheral neuropathy that prevents the sensation of discomfort associated with mechanical stress or injury to the feet. Each of these complications of diabetes increases the probability of ulcer formation on pressure-bearing areas of the feet. 15% to 20% of patients having a combination of neuropathy and vascular problems [1]. An estimated 23% of Saudis are known to have diabetes [2].

Among patients with diabetes, 15% develop a foot ulcer, and 12-24% of individuals with a foot ulcer require amputation. Treatment often requires long-term hospital admissions and frequent outpatient visits. Furthermore, loss of mobility poses a great burden on the patient and the health care system [3]. At centers of excellence, 19–35% of ulcers are reported as non healing [4-6].

Angiogenesis is a critical early aspect of the wound healing response. While hypoxia can initiate new vascularization, it cannot sustain it. On the other

hand, exposure of biological cells and tissues to pure O₂ may result in oxidative stress and genotoxicity [7].

There is no question that exposure to pure O₂ presents risks. Favorable outcomes in studies, using sub-pure O₂ under normobaric conditions [8] lead us to question the use of pure O₂ under pressure for wound therapy. It has been established that VEGF is a major long-term angiogenic stimulus at the wound site. O₂ treatment induces VEGF mRNA levels in endothelial cells and macrophages [9-11], and increases VEGF protein expression in wounds in vivo [12]. Recently it has been shown that O₂ may trigger the differentiation of fibroblasts to myofibroblasts [13], which are responsible for wound contraction.

The amount of O₂ at which collagen synthesis is half-maximal (K_m is using Micahelis-Menton equation) has been determined to occur at a pO₂ of 20 to 25 mm Hg [14, 15], with V_{max} occurring at levels approaching 250 mm Hg. This represents levels of O₂ availability that exceed the pO₂ normally present in wounds, and suggests that supplemental O₂ may enhance collagen synthesis, and process of healing.

This has, in fact, been shown to be true, both in in-vivo models and in human subjects. Increasing wound oxygenation results in increased collagen deposition and tensile strength, with maximal effects seen at levels in which wound oxygenation is increased above normal physiologic conditions by the addition of supplemental O₂ [16-18]. Hyperbaric Oxygen (HBOT) is capable of elevating arterial pO₂ as high as 1200 mm Hg.

But still areas of the wound not supported by blood vessels will not benefit as much. While topical O₂ is not likely to diffuse into deeper tissues, it does have the advantageous potential to oxygenate superficial areas of the wound not supported by intact vasculature. In this way, topical O₂ may correct pO₂ of cells at the wound core, thus correcting hypoxia induced impairment of NADPH oxidase function in those cells. NADPH oxidase function in wound-related cells contributes to favorable processes such as cell motility, angiogenesis, and extracellular matrix formation [19].

Such fine-tuning of conditions for O₂ therapy should result in more cost-effective and efficient care, minimizing barotraumas and other risks associated with use of pressurized pure O₂. If proven to be efficient, topical O₂ therapy has the added advantage of caring for a much larger potential patient population, especially under conditions of public disaster and in a field-setting where HBOT is simply not applicable.

Negative pressure wound therapy in addition of having ability to induce angiogenesis by developing a state of topical hypoxia, it may aid in the healing process by wound retraction [20], stimulation of granulation tissue formation [21], continuous wound cleansing, continuous removal of exudates [22] and reduce interstitial edema [23] that promote blood flow back into the wound area. However, some studies have noted no change or an increase in bacterial burden present in the wound [24].

In this study, we will use a mixture of the two procedures Topical pressurized oxygen therapy (TPOT) and Vacuum therapy (VAC) in a group of patients to assess the rate of healing of the foot ulcers in comparison with each individual procedure.

The aim of the study is to assess the rate of healing of diabetic foot ulcer under the effect of adjunctive treatment with low pressure, oxygen therapy (TPOT) or negative pressure wound therapy (VAC) alone or the alternating combination of the two procedures. Based on the findings, we may trace the behavior of wound healing and extrapolate the best

approach that would have a privilege therapeutic effect on chronic foot ulcers in patients with diabetes.

MATERIALS AND METHODS:

This study is a randomized, single-center, double-blinded, case controlled clinical trial that evaluated the effect of low pressure, oxygen or negative pressure irrigation alone or combination of both on ulcer healing in diabetic patients with chronic foot ulcers. The outcomes for the group receiving topical low pressure, oxygen was compared with those of the group receiving treatment with negative pressure irrigation, and a third group with a combination of both. All three groups were subdivided based on Ankle Brachial Index ABPI to good perfusion subgroup > 0.9 and poor perfusion subgroup < 0.9.

All patients had diabetes and at least one full-thickness wound below the ankle for more than 3 months. They were previously treated at a diabetes foot clinic for a period of no less than 2 months. All patients assessed by a vascular surgeon at the time of inclusion, and only patients with adequate distal perfusion included in the study. Patients having an acute foot infection will be included when the acute phase was resolved. Oral or local antibiotic treatment did not exclude patients from study participation. Cases of malignancy, and untreated thyrotoxicosis were excluded, also, current drug or alcohol misuse, vascular surgery in the lower limbs within the last two months, participate in another study, or suspected poor compliance were excluded. All participants provided written informed consent.

PROCEDURES

20 Patients have been stratified based upon arterial toe blood pressure (ABPI ≤0.9 vs. >0.9 subgroups) [25], before being randomly assigned to either of the study groups. Randomization has been done in blocks of 6 using sealed envelopes see Fig1.

The study performed in an ambulatory setting. Treatment sessions of topical oxygen were given five days per week for eight weeks (40 treatment sessions). For the VAC therapy, pressure was adjusted to -1.5 Barometric pressure, continuous suction, with replacement of the dressing every 3 days. The treatment period could be extended to 10 weeks, but the number of treatment sessions was not allowed to exceed 40. Treatment has been given as an adjunct to regular treatment at the multidisciplinary diabetes foot clinic, which included treatment of infection, revascularization, debridement, off-loading, and metabolic control according to high international

standards [26]. Investigators did not intervene in the daily routine clinical management of the patients. Outcomes were measured once every week during the treatment period (first 8–10 weeks) and then at three-month intervals. Ulcers were graded using the Wagner classification system [27], and ulcerated areas were measured using Visitrak Digital (Smith & Nephew, Hull, England). Assessment of healing surface area was done by using Visitrak digital [28].

Topical oxygen therapy applied by using an oxygen concentrator (Sequal- model REGALIA), and single use Hyper-Box Chamber Bx/20 Model: G00020) Negative pressure therapy applied by using VAC Machine, KCI corporation (<https://www.kciexpress.com/kciexpress/>).

The primary endpoint is healing of the ulcer. The ulcer was defined as the ulcer with the largest area and duration of at least two months at the time of inclusion. An ulcer was considered healed when it was completely covered by epithelial regeneration and remained so until the next visit in the study. Wagner grade 4 ulcers were considered healed when the gangrene had separated and the ulcer below was completely covered by epithelial regeneration. If a patient died during the follow-up period, he/she will be censored at the time of the death. If a major amputation (above ankle amputation) was required, the ulcer will be considered not to have healed. Secondary endpoints are major amputations and death.



Fig. 1: Topical oxygen therapy applied by using an oxygen concentrator (Sequal- model REGALIA), and single use Hyper-Box Chamber Bx/20 Model: G00020) Negative pressure therapy applied by using VAC Machine, KCI corporation.

STATISTICAL ANALYSIS:

The treatment code will not be broken until the last patient had completed the 1-year follow-up visit. Data entry and statistical analyses were performed using the Statistical Package for Social Science (SPSS) program for windows version 22. Mean and standard deviation was used for quantitative variables. P-values of <0.05 were considered statistically significant using Paired Sample T-test. Data correlation for the rate of healing at each therapy technique were done by using Bivariate Correlate level (2-tailed) where (**.

Correlation is significant at the 0.01 level (2-tailed)) and (*. Correlation is significant at the 0.05 level (2-tailed)).

RESULTS:

In this study, we tested the effect of (VAC) negative pressure therapy and (TPOT) Topical oxygen therapy and a combination of both on the rate of healing of diabetic foot ulcers. In all these three major groups, each was sub-divided based on (ABPI) ankle brachial pressure index, into subgroup with good perfusion =

ABPI > 0.9 and subgroup with poor tissue perfusion= ABPI < 0.9.

Under VAC therapy the rate of healing was $3.9900 \pm .18074 \text{ mm}^2/\text{W}$, with ABPI > 0.9. This rate of healing is significantly higher than VAC therapy with ABPI < 0.9 was $2 \pm .39805 \text{ mm}^2/\text{W}$ and ($P < 0.002$)

Under TPOT; the rate of healing by using TPOT with ABPI > 0.9 was $3.1900 \pm .18074 \text{ mm}^2/\text{W}$ and there is no significance difference of this group when compared with TPOT with ABPI < 0.9 group was $3.0750 \pm .63125 \text{ mm}^2/\text{W}$ and ($p < 0.004$)

Under the use of combined therapy Mixed groups, the rate of healing in the mixed group with ABPI > 0.9 was $5.5100 \pm .74863 \text{ mm}^2/\text{W}$ which is significantly higher than that of mixed group with ABPI < 0.9 was $4.4450 \pm .60757 \text{ mm}^2/\text{W}$ and ($P < 0.0001$)

The combined therapy (mixed groups with ABPI > 0.9), showed the high significant rate of healing when compared with corresponding groups of VAC therapy ABPI > 0.9 ($p < 0.004$) and also with TPOT ABPI > 0.9 ($P < 0.004$)

These findings are still present when comparing the mixed group with ABPI < 0.9 and VAC with ABPI < 0.9 ($P < 0.0001$), and also when compared with TPOT with ABPI < 0.9 ($P < 0.001$).

On comparing VAC groups with TPOT groups; the VAC therapy groups showed a significant higher rate of healing than TPOT groups ($p < 0.0001$) when ABPI > 0.96, but the opposite was noticed when ABPI < 0.9 in which the rate of healing under TPOT is much better than VAC therapy ($p < 0.0001$)

Table (1), show the average rate of healing and statistical analyses in all six study groups.

Pair	Mean	Std. Deviation	P-Value
VAC<0.9	2.0000	.39805	.002
VAC>0.9	3.9900	.18074	
OXY<0.9	3.0750	.63125	.004
OXY>0.9	3.1900	.18074	
MIX<0.9	4.4450	.60757	.000
MIX>0.9	5.5100	.74863	
VAC<0.9	2.0000	.39805	.000
OXY<0.9	3.0750	.63125	
VAC<0.9	2.0000	.39805	.000
MIX<0.9	4.4450	.60757	
VAC>0.9	3.9900	.18074	.000
OXY>0.9	3.1900	.18074	
VAC>0.9	3.9900	.18074	.000
MIX>0.9	5.5100	.74863	
OXY<0.9	3.0750	.63125	.001
MIX<0.9	4.4450	.60757	
OXY>0.9	3.1900	.18074	.000
MIX>0.9	5.5100	.74863	

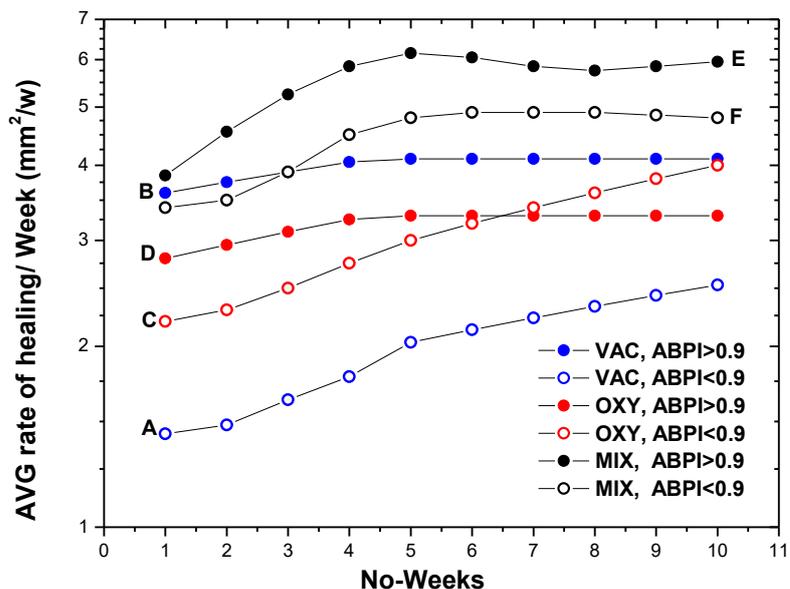


Fig. 2 The correlation between the healing response and the duration of OXY, VAC and MIX therapy



Fig. 3 Assessment of healing rate for a patient with diabetic foot ulcer.

Fig. 2A shows delay in onset of healing for the first 2 weeks, then progress steady after that under VAC

therapy with poor perfusion ABPI < 0.9 (r=.991** and P-value=.0001), while on a good perfuse limb ABPI > 0.9

($r=.822^{**}$ and $P\text{-value}=.003$) the onset of healing process started early with starting the VAC therapy and the total duration for complete healing is shorter (Fig. 2B).

On TPOT the onset of healing process started early to start of therapy (Fig.2C ($r=.999^{**}$ and $P\text{-value}=.0001$) and Fig.1D ($r=.822^{**}$ and $P\text{-value}=.003$)), but the duration of complete healing was very short in good perfuse limb (Fig.2D) and much delayed in poor perfuse limb Fig.2C).

In mixed therapy; still the onset of healing is delayed when there is poor limb perfusion (Fig.2E ($r=.856^{**}$ and $P\text{-value}=.002$)), but the duration of complete healing was shorted in either good perfusion (Fig.2F ($r=.745^{**}$ and $P\text{-value}=.002$)) or poor perfusion (Fig.2E).

DISCUSSION

The vast majority of the current literature focuses on the sensing of hypoxia, and the work on hyperoxic sensing is limited. Both hypoxia and hyperoxia are relative terms. They refer to a state of oxygenation that departs from the normoxic set point, i.e., the pO_2 to which cells or tissues adjust to under basal conditions. [29], for any given cell or tissue, normoxic set point represents that state of oxygenation where the cell or tissue does not report hypoxia neither do they induce hyperoxia-induced cell signaling or manifest overt oxygen toxicity. It is likely that this set point would represent a range of pO_2 , the span of which might depend on the tissue in question. Any change of O_2 ambience exceeding that span would result in the switching on of a hypoxic or hyperoxic response in the finest of scales [30,31].

In a wound with pockets of hypoxia ranging in magnitude from extreme to the marginal, the goal should be to reestablish normoxia in the worst affected hypoxic pockets without exposing other parts of the wound tissue to such high levels of pO_2 that would antagonize healing by hyperoxia-induced growth arrest or simply overt oxygen toxicity [32]. Moderate hyperoxia increases the appearance of new blood vessels in wounds.

Among the factors that may oppose wound healing, extreme hyperoxia causes growth arrest [33-36], and cell death by a mitochondria-dependent apoptosis pathway [37]. In addition, extreme hyperoxia does pose the threat of oxidative stress [38]. Research observations revealed that; when cells grown under standard culture conditions, if these cells are maintained

under mild hyperoxic conditions, e.g., 30% O_2 , and then brought down to 20% O_2 culture conditions they report hypoxia [39].

The key is to couple hypoxia-response signaling with conditions such as appropriate tissue oxygenation that could sustain the healing process. In response to TPOT, there is no sustained change in tissue O_2 tension much beyond the period of treatment. Both too little as well as too much oxygenation may impede the healing process. Oxygen dosing based on the specific need of a wound therefore seems prudent. These previous observations clearly indicated that therapeutic approaches targeting the oxygen sensing and redox signaling pathways are promising [40].

Based on the previous data, we hypothesized that using the combined therapy may be more beneficial than using single conjunctive therapy, by fine tuning of wound oxygenation.

Our results revealed a higher rate of healing when using alternating combined VAC and TPOT in either good perfuse limb or in a poor perfuse limb as indicated with ABPI, in addition; the total duration of complete healing showed significant shortening when using this combined therapy

These findings could be explained based on resetting the normoxia set point, using the TPOT that will raise the set point for VAC hypoxic response thus initiate angiogenesis response at the good oxygenated chronic wound.

Success in wound care may be improved by a personalized wound care therapy. The key lies in our ability to raise the normoxia set point of the wound by transient TPOT that allows the VAC angiogenesis hypoxic response to act with adequate wound oxygenation that maintain the viability of the deep pockets and allows tissue repair. Investment in bringing such capabilities to clinical practice should yield lucrative returns.

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