Original Article

# Effect of Normobaric Oxygen Therapy on Wound Healing in Patients with Minor Tissue Loss from Critical Limb Ischemia: A Randomized Clinical Trial

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***Objective:*** The present study is to evaluate the effect of normobaric oxygen therapy on wound healing in patients who presented with minor tissue loss from critical limb ischemia.

***Materials and Methods:*** This randomized controlled trial was conducted at Ramathibodi Hospital. From May 2017 to January 2018, critical limb ischemia patients with minor tissue loss were randomly assigned to be treated with or without normobaric oxygen therapy for four weeks. The primary outcome is to evaluate the wound healing by measuring the wound surface area. The secondary outcome is to evaluate the change in the transcutaneous oxygen tension at pre- and post-treatment and also complications from the treatment.

***Results:*** We assigned 28 patients with minor tissue loss into two groups (after excluding one patient from each group due to infection): 14 patients each group. The wound surface area at four-week times was smaller in intervention group (-0.06$\pm$1.25 cm$^2$) but was larger in control group (0.825$\pm$1.10 cm$^2$) without statistical significance ($p$ = 0.057). Likewise, the transcutaneous oxygen tension in the intervention group showed superior result compared to the control group (3.22$\pm$9.54 vs. -3.02$\pm$7.25 mmHg) though there was no statistical significance ($p$ = 0.059).

***Conclusion:*** Normobaric oxygen therapy may be beneficial for increasing wound healing in the critical limb ischemia patients who presented with minor tissue loss.

***Keywords:*** Critical limb ischemia, Wound healing, Oxygen therapy, Minor tissue loss

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Critical limb ischemia is the most severe form of peripheral arterial disease (PAD) and is associated with a high risk of limb loss. One of the common major manifestations is ischemic ulcers or gangrene of the forefoot or toes. The healing process is hampered by inadequate tissue perfusion, oxygenation and cellular replication[(1)].

Revascularization is the best option for limb salvage. However, some patients might be unfit, or unwilling to undergo revascularization. Treatments, such as hyperbaric oxygen therapy, and stem cell therapy, are optional in promoting wound healing[(2)].

Hyperbaric Oxygen Therapy (HBOT) is the inspiration of 100% oxygen while under increased atmospheric pressure about 2 to 3 atmospheres. The principle of this treatment is to increase the oxygen dissolved in the circulating blood plasma, especially into the area of relative hypoxia[(3)].

Additionally, Oxygen played a role in wound healing by acting as a substrate for ATP synthesis and a supply of metabolic energy, supports the differentiation of fibroblast to myofibroblast, reduces infection and also promotes the synthesis of collagen for angiogenesis and tissue remodeling.

However, in Thailand, HBOT has many limitations, such as high cost, low availability of HBO chambers, lack of trained personnel to monitor patients, poor patient compliance (headache and otalgia) and some possibility of high oxidative potential contributing to pulmonary edema and brain injury.

Due to the limitations of HBOT, we tried to find the alternative treatment, that is easy to acquire and more affordable. The treatment that fits this criteria and possibly promote wound healing in Thailand which leading to hypothesized that "Normobaric oxygen therapy might be

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some value in promoting wound healing"; NBOT is defined as breathing oxygen greater than 21%, up to 100%, at 1 absolute atmosphere pressure[4].

When compare NBOT to HBOT, NBOT has been considered to have several advantages, such as high availability, good patient compliance, low cost, low complication, few technical requirement, and may be applicable to home therapy. To the best of our knowledge, this is the first prospective, randomized trial that studied about the role of normobaric oxygen therapy in the critical limb ischemia patient.

## Materials and Methods

### *Study designs*

This open labeled randomized study was conducted at single center, Ramathibodi Hospital with approval by the ethic committee. We used block of four randomization and sealed envelopes generated in block of four. All patients provided written informed consents.

### *Selection of patients*

All of the critical limb ischemia patients from atherosclerosis who presented with minor tissue loss (Rutherford 5) were enrolled in the study. The study excluded the patients who have infection that need urgent amputation, refuse to enroll in the study or have contraindication to oxygen therapy.

There is no absolute contraindication for NBOT but for safety of the patient that enrolled to this study, we applied the contraindication from HBOT e.g. seizure disorder, chronic sinusitis, COPD, active asthma etc. to the NBOT group too.

### *Definition*

Critical limb ischemia is a manifestation of peripheral arterial disease (PAD). It includes patients with typical chronic ischemic rest pain or ischemic skin lesions, either ulcers or gangrene, and symptoms have persisted for more than two weeks. And also with one of the objective criteria such as ankle pressure less than 70 mmHg or toe pressure less than 50 mmHg or dampened or flat pulse volume recording at the metatarsal or toe[5,6].

According to the Rutherford classification of peripheral arterial occlusive disease, Minor tissue loss is defined as a nonhealing ulcer or focal gangrene beyond the transmetatarsal level with diffuse pedal ischemia[6].

### *Treatment and medical therapy*

Patients in the intervention group received oxygen therapy via oxygen mask at FiO2 1.0 for 3 hours per day and 5 days per week for a total of four weeks at the short stay unit, Ramathibodi Hospital. Both groups received wound care by experience medical personnel, risk factor control such as diabetic mellitus, hypertension and dyslipidemia as well as encouragement for smoking cessation.

During the four-week period of study; preoperative evaluation and preparation were made so that they are ready for revascularization as soon as the study was finished. This includes preoperative imaging with femoral angiography or computed tomographic angiography, medical condition evaluation with appropriate subspecialty consultation and gathering information for planning the treatment option in conference.

The regular waiting time for revascularization is 3 to 4 weeks. Therefore, the study did not affect the timing of the operation. After the study, revascularization proceded according to the standard schedule.

### *Data collection*

For demographic data, we recorded age, sex, BMI, underlying disease and baseline laboratory.

We manually traced the wound surface area by placing a transparent film over the wound and tracing the outline with a permanent marker. The process was repeated 3 times for each follow-up. Adobe Photoshop CS3 Extend™ was used to calculate the mean value of wound surface area which there was 0.37% error from Visitrak™[7,8].

The transcutaneous oxygen tension (TcPO2) of the foot was measured before, during, and after NBOT using a PF 5040 TcPO2 (PeriFlux System 5040; Perimed AB, Stockholm, Sweden) and the PeriSoft program (Perisoft for Windows 2.50; Perimed AB) to evaluate tissue oxygenation. A TcPO2 sensor was fixed on the dorsum of the foot adjacent to the ulcer with the patient in the supine position. Even if an ulcer was present on the plantar aspect, TcPO2 was always measured on the dorsum of the foot in the area directly opposite the lesion. TcPO2 levels were recorded at 44°C after a 15-min equilibration period. The initial TcPO2 level was measured with patient's breathing room air (21% oxygen). As the effects of edema and hyperemia on TcPO2 measurements remain controversial, patients with wound infections or cellulitis were excluded from this study.

NBOT was administered to the patients through a mask under a condition of 100% oxygen (oxygen flow at 13 L/min with gas overflow) supplied via a mechanical nebulizer with a constant monitoring of the TcPO2 levels until the TcPO2 level reached a stable plateau. After discontinuing the inhalation of 100% oxygen, the patients breathed room air (21% oxygen) again until the TcPO2 level reached a plateau. The TcPO2 levels before, during, and after NBO therapy

**Figure 1.** Measurement of transcutaneous oxygen tension (TcPO2) during normobaric oxygen therapy.

were compared. The times required to reach a plateau after the 100% oxygen inhalation and after discontinuation of the therapy were measured.

And during the NBOT was given, we observed the side effect or complication.

### Sample size

To the best of our knowledge, there is no previous study about the effect of NBOT on wound healing in critical limb ischemia. There is a study about the reduction of ulcer area from HBOT compared to supportive care which was 42.4±20% vs. 18.1±6.5% ($p<0.05$)[9]. So we suspected the result from NBOT might be 25% less than the effect from HBOT and we also estimated in case of loss follow-up 20%. After calculation with the formula below (RCT for continuous data), the sample size was 23 patients per group.

$$n_{trt} = \frac{(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \left[\sigma_{trt}^2 + \frac{\sigma_{con}^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_{con}}{n_{trt}}, \Delta = \mu_{trt} - \mu_{con}$$

### Follow-up

We followed-up patients every week to measure the wound surface area and transcutaneous oxygen tension at the vascular laboratory at the OPD for four weeks.

### Study end point

The primary end point of this study is to evaluate the effect of NBOT on wound healing by measuring the change in wound surface area.

The secondary outcome is to monitor the transcutaneous oxygen tension before and after treatment, and also observe the complication during NBOT.

### Follow-up and statistical analysis

Results are given as mean ± SD and median and range when values were normal or not normally distributed respectively. Student's t-tests were used for comparison within and between groups for continuous data. Chi-square was used for comparison categorical data.

The study was designed with a power of 80% to detect differences in primary outcomes. Differences between the two groups were considered statistically significant at values of $p<0.05$. All calculations were carried out using STATA 14.

## Results

From May 2017 to January 2018, 30 patients who visit at OPD of vascular unit at Ramathibodi Hospital were randomly assigned into 2 groups; 15 patients in the intervention group and 15 patients in the control group. After excluding one patient from each group due to infection, there were 14 patients for each group to be analyzed.

Both groups were well matched at baseline with similar demographics, location of disease, clinical presentations, comorbidities, laboratories as well as wound surface area and $TcPO_2$ (Table 1).

### Primary endpoint

Overall wound surface areas after four weeks in the intervention group tend to be better than the control group (Figure 2).

The change in wound surface area after the study completion at the fourth week was compared with the baseline. The result showed that the wound surface area got smaller in the intervention group (-0.06±1.25 $cm^2$) but got larger in the control group (0.825±1.10 $cm^2$) without statistical significance ($p = 0.057$) (Figure 3).

### Secondary endpoint

Figure 4 shows the change in $TcPO_2$ of patients in the intervention group before, during and after they were breathing oxygen. $TcPO_2$ increased while they were receiving NBOT.

After finishing study at fourth week, the change in the transcutaneous oxygen tension at baseline in the intervention group show superior result compared to the control group (3.22±9.54 vs. -3.02±7.25 mmHg) though there was no statistical significance ($p = 0.059$) (Figure 5).

No complication from NBOT was found during this study.

## Discussion

Normobaric oxygen therapy is a non-pharmacological and non-invasive treatment that elevates oxygen level in the blood. Because oxygen saturation of hemoglobin in healthy individuals at sea level already reaches 95% and above, NBO cannot further increase the amount of oxygen bound to hemoglobin. Therefore, the proportion of physically dissolved oxygen becomes more relevant. Given that the Bunsen solubility coefficient of oxygen in the blood at 37°C is 0.003 mL $O^2$/mmHg/mL blood, it was reported that NBO could increase the arterial partial pressure of oxygen ($pO^2$) from a mean of 84.1 to 345 mmHg (1 mmHg = 0.133 kPa) in the aorta, and from 60.9 to 154 mmHg in the smallest arterioles[10]. Inhaling 100% oxygen at 45 L/min can raise the physically dissolved oxygen fraction from 0.3 mL to about 2 mL per 100 mL of blood[4,11].

There are some studies that applied NBOT to treat acute ischemic stroke and diabetic foot. Kyung Chul Moon, et al[12] has studied about the effect of NBOT on tissue oxygenation in diabetic feet which also has ischemic component. They concluded that NBOT significantly augments tissue oxygenation of diabetic feet and may reinforce wound healing potential. This trial has used only $TcPO_2$ as a parameter to monitor but not study the correlation to the wound surface area.

Our study has paid attention to critical limb ischemia patients and the result showed that NBOT may be beneficial in decreasing the wound size and increasing $TcPO_2$ without complication from oxygen therapy although it is not statistically significant. This might be the pilot study

**Table 1.** Baseline data

| | Intervention (14) | 95% CI | Control (14) | 95% CI | *p*-value |
|---|---|---|---|---|---|
| Sex | | | | | 0.127 |
| Male (%) | 10 (71.43) | | 6 (42.86) | | |
| Female (%) | 4 (28.57) | | 8 (57.14) | | |
| Age (year) | 71.0±9.01 | 65.87 to 76.27 | 71.5±12.64 | 64.20 to 78.79 | 0.919 |
| BMI (kg/m$^2$) | 23.24±4.65 | 20.55 to 25.93 | 22.73±3.26 | 20.85 to 24.61 | 0.737 |
| Lesion | | | | | |
| Aortoiliac | 2 (14.28) | | 5 (35.71) | | 0.190 |
| Femoropopliteal | 9 (64.29) | | 7 (50) | | 0.445 |
| Below the knee | 8 (57.14) | | 7 (50) | | 0.705 |
| Presentation | | | | | 0.541 |
| Ulcer (%) | 13 (92.86) | | 12 (85.71) | | |
| Gangrene (%) | 1 (7.14) | | 2 (14.29) | | |
| Wound surface area (cm$^2$) | 2.45±1.60 | 1.52 to 3.38 | 2.36±1.43 | 1.53 to 3.19 | 0.877 |
| TcPO$_2$ (mmHg) | 20.93±11.89 | 17.06 to 27.79 | 18.03±8.12 | 13.34 to 22.73 | 0.459 |
| Comorbidities | | | | | |
| DM (%) | 11 (78.57) | | 10 (71.42) | | 0.663 |
| HT (%) | 12 (85.71) | | 11 (78.57) | | 0.622 |
| DLP (%) | 6 (42.85) | | 6 (42.85) | | 1.000 |
| CKD (%) | 3 (21.42) | | 3 (21.42) | | 1.000 |
| ESRD (%) | 5 (35.71) | | 6 (42.85) | | 1.000 |
| CAD (%) | 5 (35.71) | | 6 (42.85) | | 0.699 |
| CVD (%) | 3 (21.42) | | 2 (14.28) | | 0.622 |
| Laboratories | | | | | |
| FBS (mg/dL) | 122.5 | 71 to 257 | 104.5 | 79 to 243 | 0.161 |
| HbA1c (%) | 6.96±1.80 | 5.92 to 8.00 | 6.72±1.36 | 5.91 to 7.54 | 0.703 |
| Cr (mg/dL) | 1.33 | 0.68 to 13.53 | 1.11 | 0.4 to 12.62 | 0.705 |
| GFR | 44.84±34.98 | 24.6 to 65.04 | 49.62±39.98 | 26.54 to 72.70 | 0.739 |
| Albumin | 31.09±5.06 | 28.03 to 34.15 | 30.25±5.02 | 27.22 to 33.28 | 0.675 |
| Cholesterol (mg/dL) | 148 | 79 to 221 | 145 | 108 to 319 | 0.835 |
| LDL (mg/dL) | 87.91±42.46 | 60.94 to 114.89 | 108.33±12.64 | 80.52 to 136.14 | 0.259 |
| HCT (%) | 33.35 | 25.1 to 42.9 | 30.9 | 27 to 48.8 | 0.059 |

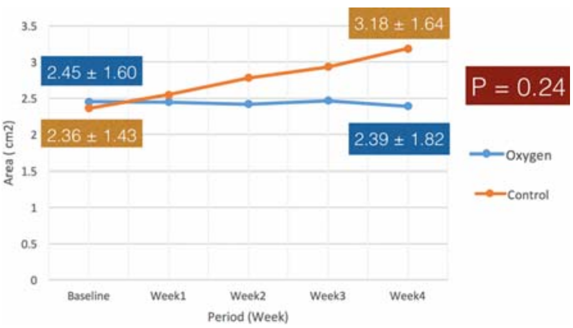


**Figure 2.** Overall wound surface area (cm$^2$) during study.

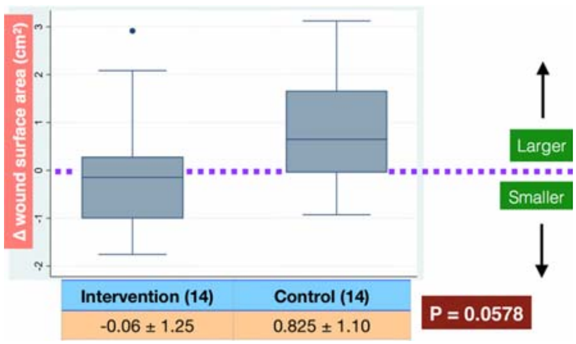


**Figure 3.** Change in wound surface area (At 4$^{th}$ week - At baseline).

with the power of 74.6%. This study still has some limitations such as small sample size and too short a study period. Greater beneficial effects or more positive outcomes might be obtained from al larger sample size, extended period of study, higher flow rates of oxygen, increase oxygen duration and other oxygen delivery devices such as reservoir or high flow.

From TASC II guidelines, revascularization is still the best option treating this condition in a good risk patient. Hyperbaric oxygen therapy should be considered in selected patients with ischemia ulcers who have not responded to, or are not candidates for revascularization[6].

Due to being previously mentioned before about the limitations of HBOT in Thailand and several advantages of NBOT (e.g. simplicity, easy to apply; can be performed

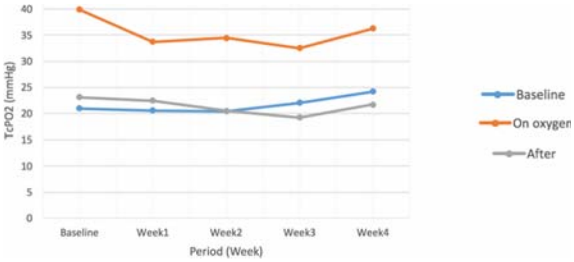


**Figure 4.** Change in $TcPO_2$ during normobaric oxygen therapy.

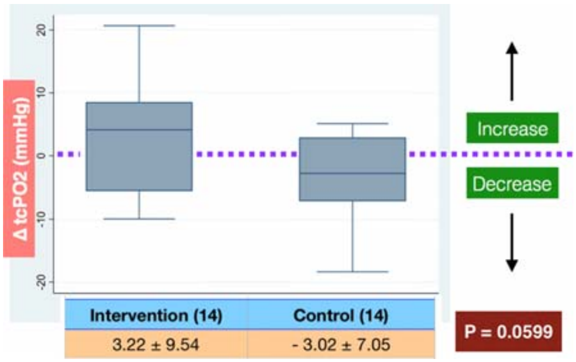


**Figure 5.** Change in trascutaneous oxygen transion (At 4th week - At baseline).

even in rural hospitals or at home, no complications and low cost), NBOT may be another option to use as an adjunctive therapy while the patient is waiting for the operation or may be used in unfit patient that could not undergo surgery.

## Conclusion

Normobaric oxygen therapy may be beneficial to increasing wound healing and transcutaneous oxygen tension in patients with critical limb ischemia who presented with minor tissue loss.

## What is already known on this topic?

Oxygen is the essential substance for the wound healing process.

Oxygen is delivered to the tissue via the carrier (Haemoglobin) in Erythrocytes and dissolution in the plasma.

The oxygen delivery to the wound, via dissolution mechanism, could be enhanced by increasing the fraction of inspired oxygen (FiO2) and ambient Pressure.

Nowadays Hyperbaric oxygen therapy is a proven-alternative option for patients with critical limb ischemia who aren't the candidate for revascularization, whereas the evidence of Normobaric oxygen therapy is still unprovided.

## What this study adds?

Even not statistically significant, this study shown that the normobaric oxygen therapy may add-on the advantage of wound healing and angiogenesis for the critical limb ischemia patients who aren't the candidate for revascularization and unable to provide the Hyperbaric oxygen therapy. However further studies are need to prove this assumption.

## Potential conflicts of interest

The authors declare no conflict of interest.

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