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Adjunctive Hyperbaric Oxygen Therapy for Healing of Chronic Diabetic Foot Ulcers

A Randomized Controlled Trial

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ABSTRACT

PURPOSE: The purpose of this study was to compare the effect of standard wound care with adjunctive hyperbaric oxygen therapy (HBOT) to standard wound care alone on wound healing, markers of inflammation, glycemic control, amputation rate, survival rate of tissue, and health-related quality of life in patients with diabetic foot ulcers (DFUs).

DESIGN: Prospective, randomized, open-label, controlled study.

SUBJECTS AND SETTING: The sample comprised 38 patients with nonhealing DFUs who were deemed poor candidates for vascular surgery. Subjects were randomly allocated to an experimental group (standard care plus HBOT, n = 20) or a control group (standard care alone, n = 18). The study setting was a medical center in Kaohsiung City, Taiwan.

METHODS: Hyperbaric oxygen therapy was administered in a hyperbaric chamber under 2.5 absolute atmospheric pressure for 120 minutes; subjects were treated 5 days a week for 4 consecutive weeks. Both groups received standard wound care including debridement of necrotic tissue, topical therapy for Wagner grade 2 DFUs, dietary control and pharmacotherapy to maintain optimal blood glucose levels. Wound physiological indices were measured and blood tests (eg, markers of inflammation) were undertaken. Health-related quality of life was measured using the Medical Outcomes Study 36-Item Short Form.

RESULTS: Complete DFU closure was achieved in 5 patients (25%) in the HBOT group (n = 20) versus 1 participant (5.5%) in the routine care group (n = 18) ($P = .001$). The amputation rate was 5% for the HBOT group and 11% for the routine care group ($\chi^2 = 15.204$, $P = .010$). The HBOT group showed statistically significant improvements in inflammation index, blood flow, and health-related quality of life from pretreatment to 2 weeks after the last therapy ended ($P < .05$). Hemoglobin A_{1c} was significantly lower in the HBOT group following treatment ($P < .05$) but not in the routine care group.

CONCLUSIONS: Adjunctive HBOT improved wound healing in persons with DFU. Therapy also reduced the risk of amputation of the affected limb. We assert that at least 20 HBOT sessions are required to be effective.

KEY WORDS: Clinical trials, Diabetes mellitus, Diabetic foot ulcers, Hyperbaric oxygen therapy, Nonhealing wounds, Nursing care, Quality of life.

INTRODUCTION

Foot ulcers and infections are prevalent in persons with diabetes mellitus; reported prevalence rates vary from 1.7% to 25%.[1-3] Approximately 40% of patients with diabetic foot infections return to hospital for repeated treatment, and 1 in 6 of these individuals will die within 1 year.[4] The pathophysiology of diabetic foot ulcers (DFUs) is not entirely understood. Individuals with diabetes mellitus often have impaired leukotaxis and phagocytosis function that increase the likelihood of developing a wound infection by 17-fold.[5] A nonhealing, infected DFU damages both soft tissue and bone; 85% of individuals who develop a DFU ultimately undergo amputation, and 68% will die within 5 years of amputation.[6-8] The severity of the ulcer, angiopathy, infection, and poor blood glucose control are important predictors for diabetic foot amputation.[9-11]

Hyperbaric oxygen therapy (HBOT) is used as adjuvant therapy in conjunction with topical and systemic therapy frequently including debridement, recombinant human platelet-derived growth factor, or other skin substitutes in persons with nonhealing or deteriorating DFU.[12] Evidence concerning the efficacy of HBOT for healing DFUs is mixed. Some researchers report greater efficacy when HBOT is compared to sham or placebo treatment,[13-16] but others found no differences.[17-19] Hyperbaric oxygen therapy also promotes resolution of infection in persons with DFU, and it reduces the likelihood of amputation.[14,15]

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Despite mixed evidence concerning its efficacy, HBOT has been advocated and adopted for DFU treatment in some wound care centers. The aim of this study was to compare the effect of HBOT plus standard wound care to standard wound care alone in the treatment of DFU. Outcome measures were wound size, amputation rate, survival rate of tissue in the affected limb, markers of inflammation, glycemic control, bacterial colonization (distribution of microorganism culture isolated from the wound), and health-related quality of life (HRQOL).

METHODS

We conducted a prospective, randomized, controlled trial to evaluate the efficacy of HBOT plus standard therapy to standard therapy alone for treatment of DFUs. Data were collected from June 2011 to June 2013. The time frame for data collection was divided into following 4 segments: pretreatment (before first administration of HBOT; T1), during treatment (at the 10th administration of HBOT; T2), posttreatment (at the 20th administration of HBOT; T3), and follow-up (2 weeks after the last therapy ended; T4). The study setting was a medical center in Kaohsiung City, Taiwan.

Inclusion criteria were (1) adults 20 years or older, (2) diagnosis of diabetes mellitus, (3) nonhealing DFUs that had not achieved closure after at least 2 months and following treatment for at least 1 month, (3) Wagner wound classification of grade 1, 2, and 3 ulcers, and (4) deemed appropriate for hospital admission because of skin ulcer and soft-tissue infection. Exclusion criteria were (1) gangrene, (2) contraindication for HBOT such as untreated pneumothorax, active cancerous condition, chronic obstructive pulmonary disease, and pulmonary emphysema with retention of CO_2, and (3) planned vascular surgical procedures or revascularization of the limb.

Study procedures were reviewed by the institutional review board of the Chang Gung Medical Foundation (IRB1010507C; No. 100-0876B and 101-0507C). This trial was registered at https://clinicaltrials.gov/ under the identification code NCT02328508. We ensured patients' rights throughout the study according to the ethical principles for medical research on human beings set out in the Declaration of Helsinki. All participants provided written informed consent prior to random allocation to the control or intervention group.

Sample Size

Sample size was based on a power analysis using the Statistical Software Sample Power software, version 2.0. The power was set at 0.8 to limit the risk of committing a type II error to 20%, the α level was set at .05, and the covariate's R^2 at 0.13. The sample size was calculated based on a previous clinical trial of professionally lead support group for Taiwanese people with schizophrenia in 4 waves of data collection (baseline, 10, 20, and 30 days of HBOT).[20] Therefore, the effect size of covariate adjustment in this study was set at 0.37, and the number of samples for each group was calculated to be 20. Based on these calculations, a total of 40 patients would be needed for 4 waves of data collection in order to provide 80% power (2-sided $P < .05$) to detect statistically significant differences (P value of .05) between 2 groups, at moderate effect sizes of 0.68 and 0.70, respectively, and power of 0.8 to account for a 15% attrition rate.[21,22] In this study, 38 patients were selected and randomly allocated to 2 study groups.

Study Procedures

Patients were randomly allocated to HBOT plus routine care or routine care alone groups. Group allocation was randomly generated by a computer and sealed by the primary researcher in opaque, serially numbered envelopes. Another researcher enrolled participants and assigned participants to interventions. Patients in both the intervention (HBOT) and control (routine care only) groups were hospitalized during the study, which reduced variability in standard care (Figure 1).

Standard care incorporated topical and systemic therapy for DFUs. It included maintaining good blood glucose control, offloading, debridement of necrotic tissue, antibiotic therapy for management of diabetic foot infection, and topical dressings, depending on the type and grade of the ulcer. An array of wound dressings was used for topical therapy. For example, silver-impregnated dressings such as Flamazine ointment (silver sulfadiazine) were applied to Wagner grade 1 and 2 DFUs. Hydrocolloid dressings, topical amoxicillin, and hydrogels were applied for Wagner grade 2 and 3 DFUs; wound dressings and topical piperacillin/ciprofloxacin were used to manage Wagner grade 3 and 4 DFUs. Antibiotic therapy was driven by protocol and culture and sensitivity reports.

Hyperbaric oxygen therapy was delivered via a multiperson chamber. Patients allocated to the HBOT group were placed in a hyperbaric chamber daily 5 days per week for 4 consecutive weeks for a total of 20 sessions. Patients were treated with 2.5 absolute atmospheric pressure for 120 minutes. The time period of the intervention was approximately 1 month. The intervention program was carried out by the primary research team (C.-Y.C. and R-W.W.).

Outcome Measures

We selected multiple outcome measures to evaluate the efficacy of HBOT as adjunctive therapy for patients with nonhealing DFU. They included wound physiological indices and blood biochemistry tests. Data were collected at 4 different time frames from T1 to T4. The same outcome measures were collected at the same time frames for both groups. Wound assessment was based on the Wagner classification system.[23,24] The Wagner grading system considers depth of penetration, the presence of underlying osteomyelitis, and the extent of tissue necrosis; ulcers are graded from 0 (preulcerous changes) to 5 (amputation required). Patient wounds classified as Wagner grade 0 or 1 were considered healed.[25]

Serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were used to evaluate inflammation. Erythrocyte sedimentation rate was measured with an ESR instrument (model Bedia-15s; Becton, Dickinson and Company, Taipei City, Taiwan). C-reactive protein levels were measured via a Unicel automatic biochemistry analyzer (Beckman, Brea, California).

Foot ulcer microbiology and presence of infection were evaluated via wound cultures obtained using the aseptic swab technique[26] and were collected for anaerobic and aerobic bacterial cultures. Antibiotic therapy was based on culture and sensitivity findings.

Blood flow perfusion scan was used to show when blood flow started and to evaluate tissue survival rate[27] in the affected limb during the study period. Measurements were performed using the PeriScan PIM II Laser Doppler Perfusion Imager (Perimed, Beijing, China) by R-W.W. This system visualizes spatial blood perfusion by scanning across the tissue in the

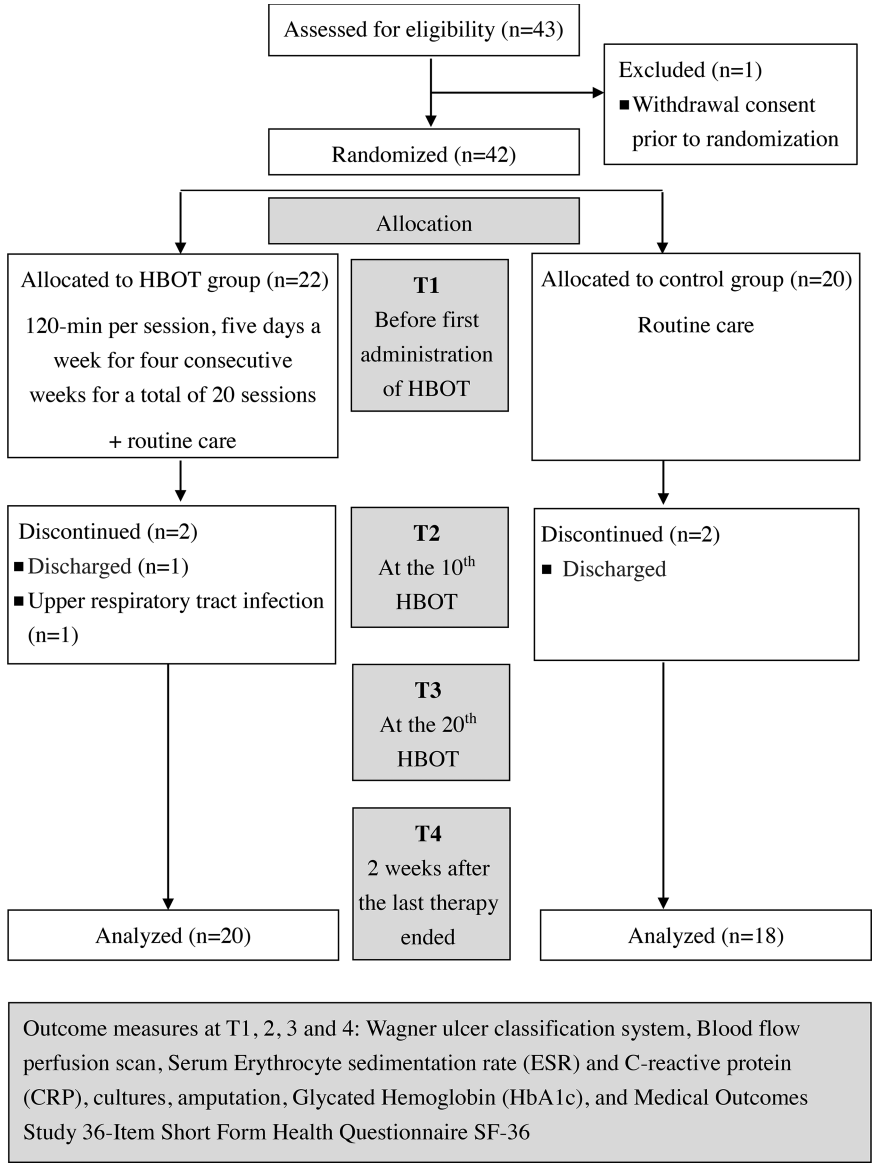

Figure 1. Flow diagram of study procedures. HBOT indicates hyperbaric oxygen therapy.

affected limb over time. The test value is set at 0 to 5 V. The detected signal is measured to extract information about local microcirculatory blood flow. Patients' attending physician discussed the data with a vascular surgeon and our research team and finally decided the treatment plans or effects. Vascular status and the need for amputation were evaluated by a vascular surgeon who determined whether amputation was necessary based on several criteria such as severe systemic infection, Wagner grade 4 DFU (partial foot gangrene), or grade 5 DFU (whole foot gangrene).[28]

Glycated hemoglobin (HbA_{1c}) was measured to assess glycemic control. Value higher than normal range (4% and 5.6%) indicated that the patient's average 3-month blood glucose value is high. The HbA_{1c} tests were done in the morning and performed using a PRIMUS HbA_{1c} analyzer manufactured by the Progressive Group Inc (Taipei City, Taiwan) and Trinity Biotech USA Inc in Kansas City.

The Medical Outcomes Study 36-Item Short Form Health Questionnaire (SF-36), Taiwan version,[29] was used to evaluate HRQOL. The SF-36 is a well-validated instrument used to measure HRQOL that yields 2 summary scores, a Physical Component Summary (PCS) and a Mental Component Summary (MCS).[30] The Cronbach α for the 8 subscales was greater than 0.74.[31] SF-36 has been used to measure HRQOL in patients with DFU in at least 2 prior studies evaluating the efficacy of HBOT.[13,32] Higher scores represent better physical and mental functioning. The SF-36 uses norm-based (50 ± 10, mean ± SD) scoring methods; for example, an individual respondent's scale score is lower than 45, meaning health status is lower than the average range. With norm-based scoring, differences in scale scores may reflect the impact of the disease or other discomforts.

Data Analysis

Data were analyzed using the SPSS software program, version 20 (Statistical Package for Social Sciences, Chicago, Illinois). Categorical data were evaluated using the χ^2 test for homogeneity. Not all continuous variables were normally distributed,

and they were analyzed using the Mann-Whitney *U* test. Specifically, the Mann-Whitney *U* test was used to evaluate differences in CRP, ESR, HbA_{1c}, and Doppler measurements. Kruskal-Wallis *H* tests analyzed changes in the CRP, ESR, HbA_{1c}, and Doppler measurements between the 2 groups before and after the HBOT. The trends of change over time of the SF-36 were compared between groups by generalized estimating equation (GEE) using the first-order autoregressive (AR1) to handle repeated observations within the subject. A proper working correlation matrix when applying the GEE method. Statistical significance was set at $P < .05$.

RESULTS

Thirty-eight patients completed the study, with 20 in the HBOT group and 18 in the routine care group. The mean age ± SD of patients in the HBOT and routine care groups was 64.3 ± 13 and 60.8 ± 7.2 years, respectively. The distributions of age were compatible between the 2 groups. Similarly, all other demographic variables of patients in the routine care group were compatible to those in the HBOT group ($P > .05$). At baseline, no significant differences in demographic and clinical characteristics were noted between the HBO and control groups following random allocation (Table 1).

Baseline evaluation found no differences in wound severity between the groups ($\chi^2 = 1.643$, $P = .200$). When assessed at 2 weeks after the individual's last therapy ended (T4), 3 patients in the HBOT group had Wagner grade 1 wounds, 7 had grade 2 wounds, 4 received skin grafts, 5 healed, and 1 underwent amputation. In contrast, 9 patients had Wagner grade 3 wounds, 3 had grade 2 wounds, 1 had grade 1 wound, 2 patients received skin grafts, 1 patient healed, and 2 patients underwent amputation. This difference in wound severity following treatment was statistically significant ($\chi^2 = 15.204$, $P = .010$).

Wound-healing scores differed significantly between the interventio and control groups at the 20th administration of HBOT (T3) to T4 ($Z = -4.205$, $P = .038$, Mann-Whitney *U* test). Wounds in the HBOT group began to show clear improvements at T3. We also quantified wound healing based on a score of 0 to 5, where a score of 5 indicated a healed wound, a score of 4 indicated wound managed by skin grafts, a score of 3 indicated a Wagner grade 1 wound, a score of 3 indicated a Wagner grade 2 wound, a score of 2 indicated a Wagner grade 3 wound, and a score of 0 indicated amputation; this scoring system is used in Figure 2A to illustrate the effect of HBOT on ulcer healing.

Differences over time were analyzed by the Kruskal-Wallis *H* test; analysis revealed a significant decrease between the ESR value at T4 versus T1 in the HBOT group ($Z = -3.291$, $P < .001$; Table 2). There were no significant differences in the routine care group ($Z = -1.743$, $P > .05$). The ESR values at T4 in the HBOT group were significantly lower than those in the routine care group by using the Mann-Whitney *U* test ($Z = -4.096$, $P < .05$). Degree of changes in ESR values from T1 to T4 is shown in Figure 2B.

Analysis also revealed significantly different CRP values between T4 and T1 in the HBOT group (Kruskal-Wallis *H* test, $Z = -3.920$, $P < .05$; Table 2). Participants in the HBOT group had significantly lower CRP levels at T4 when compared to subjects in the routine care group ($Z = -3.480$, $P < .001$; Figure 2C). We found a significant decline in mean HbA_{1c} levels at T4 in the HBOT group (Kruskal-Wallis *H* tests, $Z = -3.826$, $P < .001$), but no differences were found in the routine care group (Table 2). Degree of changes in HbA_{1c} values at T1, T3, and T4 can be seen in Figure 2D.

Doppler blood flow in the limb with a DFU significantly increased at the 10th administration of HBOT (T2) and maintained that level ($Z = -2.221$, $P < .05$; Table 2). In contrast, no significant change in blood flow was observed in the control group.

In the HBOT group, *Proteus mirabilis* (*P mirabilis*), *Staphylococcus aureus* (*S aureus*), and *Morganella morganii* appeared at T1. *S aureus* and *P mirabilis* persisted from T1 to T4. There was a striking decrease in the number of pathogens of *S aureus* and *P mirabilis* per sample from 5 to 2 and 7 to 1, respectively. *Morganella morganii* disappeared at T2. In the routine care group, new species, *Pseudomonas aeruginosa*, appeared at T2 and showed an increase in the number of pathogens per sample from 4 (T2) to 7 (T4).

Scores from the SF-36 questionnaire were analyzed using the GEE, taking into account mean values and statistically significant difference between 2 groups for each subscale (Figures 3A-3H). Improvements were noted after 20 HBOT sessions (T3) and persisted at posttreatment follow-up (T4). Improvement was noted on both PCS and MCS. Using a mixed linear model analysis, the HBOT group ($F = 24.297$, $P < .001$) had significant progress in PCS at all time points, compared to the routine care group ($F = 1.661$, $P = .171$), and in MCS ($F = 11.195$, $P < .001$ vs $F = 2.491$, $P = 0.052$).

DISCUSSION

Findings from our study suggest that HBOT, when combined with standard care, alleviates inflammatory indices of persons with nonhealing DFU. Specifically, we examined the effect of HBOT at 4 points in time; wounds in the routine care plus HBOT showed signs of wound healing after 10 treatments (T2), while wounds in the routine care group began to deteriorate. As shown in Figure 2A, the status of wounds in patients with HBOT was significantly improved at the end of treatment (T3) and improved further at follow-up (T4), whereas DFUs in the routine care group showed little change throughout the study. Our results are consistent with prior studies reporting that adjunctive HBOT reduced wound size.[13,33,34]

Changes in ESR and serum CRP levels in patients with DFU are particularly relevant because of their utility in evaluating treatment efficacy and likelihood of amputation.[1,15,35,36] We found that patients allocated to adjunctive HBOT experienced a reduction in the inflammatory marker ESR (Table 2, Figures 2B and 2C). These changes were not seen or were less prominent in the routine care group, supporting findings from others that HBOT can reduce inflammation in diabetic foot wound tissue.[37] In type 2 diabetes, low-grade inflammation is also reflected in serum CRP levels.[1,36] Natorska and colleagues[36] found that CRP concentrations in 40 diabetic and nondiabetic patients with aortic valve stenosis were 9.20 and 4.70 mg/L, respectively. The white blood cell concentration in blood and ESR have been found to be significantly elevated in patients with DFU and infection requiring amputation.[35] The relationship between inflammation and wound healing is not entirely understood; it may be attributable to the actions of several bioactive chemicals. Hyperbaric oxygen therapy has been shown to suppress multiple cytokines, such as interleukin-1 (IL-1) and interleukin-6 (IL-6), that trigger a local inflammatory response and suppress tissue necrosis factor-alpha

TABLE 1.
Demographic and Pertinent Clinical Characteristics of the Intervention and Control Groups[a]

	HBOT + Routine Care Group (N = 20)	Routine Care–Only Group (N = 18)	*P*
Age[b]	64.3 ± 13.0	60.8 ± 7.2	.254
Years living with DM[b]	13.7 ± 6.5	14.6 ± 6.6	.66
Days with DFU[b]	59.1 ± 48.8	34.9 ± 33.6	.053
Days of wound treatment[b]	34.5 ± 39.7	22.3 ± 24.9	.16
Sex[c]			.36
Male	10 (50)	11 (61.1)	
Female	10 (50)	7 (38.9)	
Marriage status[c]			.07
Single	10 (50)	4 (22.2)	
Married	10 (50)	14 (77.8)	
Education[c]			.33
Junior high school or lower	14 (70)	15 (83.3)	
High school or higher	6 (30)	3 (16.7)	
Religious beliefs[c]			.63
Yes	13 (65)	13 (72.2)	
None	7 (35)	5 (27.8)	
Occupation[c]			.20
Employed	4 (20)	7 (38.9)	
Unemployed	16 (80)	11 (61.1)	
Income[c]			.92
≤TWD $15,000[d]	9 (45)	9 (50)	
TWD $15,001-29,999[d]	8 (40)	7 (38.9)	
≥TWD $30,000[d]	3 (15)	2 (11.1)	
Living arrangment[c]			.94
Nuclear family	10 (50)	10 (55.6)	
Composite family	6 (30)	5 (27.8)	
Single	4 (20)	3 (16.7)	
Primary caregiver[c]			.08
Family	18 (90)	12 (66.7)	
(Foreign) domestic worker	2 (10)	6 (33.3)	
Medical history[c]			.14
Yes	10 (50)	13 (72.2)	
No	10 (50)	5 (27.8)	
Hypertension[c]			.52
Yes	8 (40)	8 (44.4)	
No	12 (60)	10 (55.6)	
Cardiovascular disease[c]			.11
Yes	4 (20)	…	
No	16 (80)	18 (100)	
Renal disease[c]			.29
Yes	4 (20)	7 (38.9)	
No	16 (80)	11 (61.1)	
Cerebrovascular disease[c]			.54
Yes	2 (10)	1 (5.6)	
No	18 (90)	17 (94.4)	

Abbreviations: DFU, diabetic foot ulcer; DM, diabetes mellitus; HBOT, hyperbaric oxygen therapy.
[a]Data are shown as mean values ± standard deviation. Numbers in parentheses are percentages unless indicated otherwise.
[b]Examined by the Mann-Whitney *U* test.
[c]Examined by the χ^2 test.
[d]USD vs TWD (US dollar to Tiawan dollar) exchange rate ≒ 1 vs 30.1.

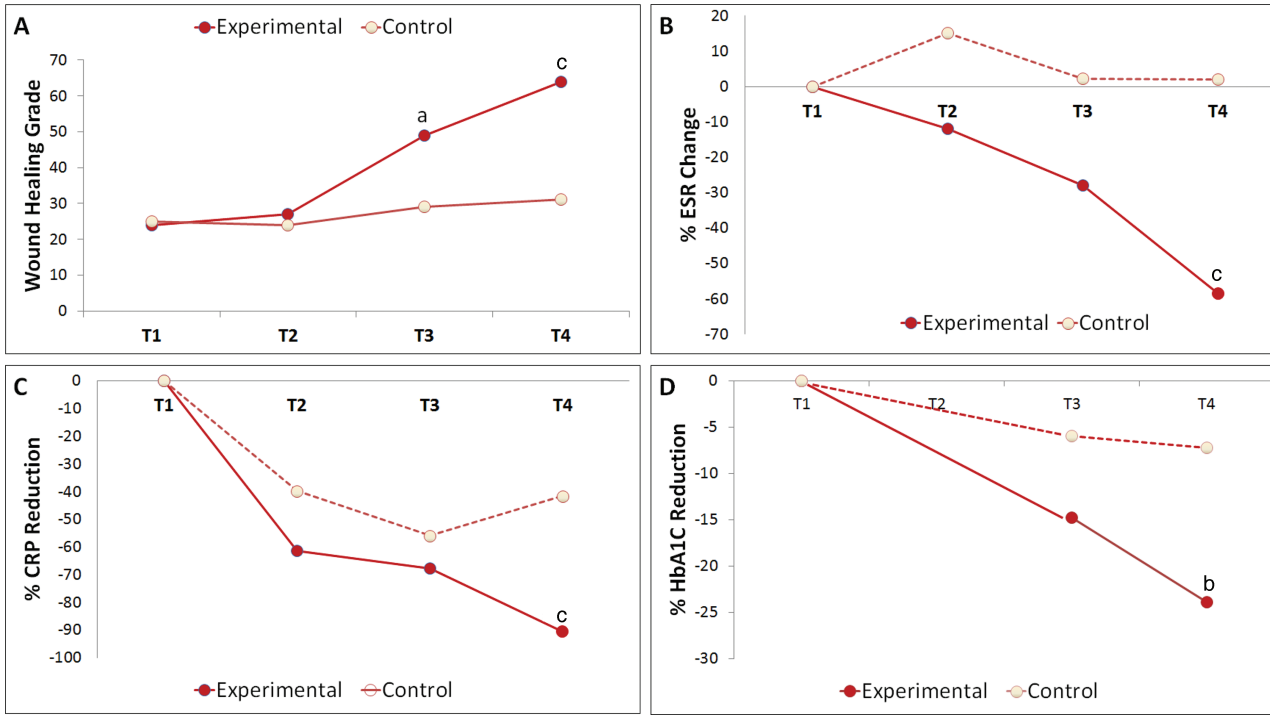

Figure 2. Effect of HBOT on wound healing (A), % ESR change (B), % CRP change (C), and % HbA_{1c} reduction (D). [a]$P < .05$; [b]$P \leq .01$; [c]$P \leq .001$. (A) Wound healing classification (based on Table 2) was quantified as follows: heal, 5; skin graft, 4; grade 1 wound, 3; grade 2 wound, 2; grade 3 wound, 1; amputation, 0. Higher scores represent better wound situations. (B-D) % change of ESR, CRP, and HbA_{1c} was calculated on % change from T1 (Table 2). —●—, HBOT group; …o…, routine care group. ESR indicates erythrocyte sedimentation rate; CRP, C-reactive protein; HbA_{1c}, glycated hemoglobin; and HBOT, hyperbaric oxygen therapy.

(TNF-α), which induces tissue death, which can stimulate the anti-inflammatory cytokines such as interleukin-10 (IL-10).[36]

Patients managed by HBOT also experienced reductions in serum levels of CRP, and the magnitude of improvement was proportional to the number of HBOT treatments (Table 2). Analysis also revealed that the percentage of improvement in CRP levels in patients allocated to adjunctive HBOT was 38.95%, 49.73%, and 78.11%, respectively, at T2 to T4. In contrast, the improvement in control group patients receiving routine care from T1 to T4 was 18.72%. These findings are consistent with those of Wunderlich and colleagues,[34] who reported that 48% to 95.2% of patients who underwent an average of 12 to 50 HBOT treatments experienced increased wound healing and reduced wound severity.

The amputation rate was significantly lower in patients allocated to routine care plus HBOT (5% vs 11%, $P = 0.010$). Van Acker and colleagues[38] estimated a 2% rate for patients with Wagner grade 1 and 2 DFUs, rising to approximately 30% for grade 3 DFUs and 52% for grade 4 DFUs. Faglia and associates[39] found that amputation rates were lower in patients receiving adjunctive HBOT than in patients randomized to standard care (8.6% vs 33.3%, $P = .016$).

Hemoglobin A_{1c} levels are used to identify average plasma glucose concentrations over prolonged periods of time in patients with type 2 diabetes mellitus, and blood glucose levels were used to provide a snapshot at a single point in time.[40,41] Table 2 and Figure 2D show that a significant decrease in HbA_{1c} was found at T4 in the HBOT group ($P < .001$) but not in the routine care group. A previous study indicated that after HBOT, the average blood glucose level of patients with diabetic foot decreased an average of 50 mg/dL, and a second study shows that mean blood glucose levels fell by an average of 48 mg/dL (22.4%) following 20 HBOT sessions to a mean values of 149 mg/dL.[42,43] We found that the mean serum HbA_{1c} levels declined by an average of 15.09% at T3 and an average of 23.05% at T4. Although we found that HbA_{1c} improved upon completion of HBOT, further studies are needed to determine whether HBOT improved blood glucose control.

Both groups had poor blood supply to the wound before treatment. After undergoing 10 HBOT sessions (T2), the blood flow to the affected limb significantly improved at the end of 20 sessions and at follow-up assessment (T3 and T4). We found no clear signs of improvement of blood flow to the affected limb in the routine care group. Several mechanisms may account for this improvement; HBOT increases oxygen content in the tissue, which is attributed to improving the survival rate of tissue in the affected limb.[44] Specifically, HBOT has been attributed to transiently increasing the amount of oxygen within wounded tissue by alleviating local tissue hypoxia, improving local blood flow, and promoting healing.[45,46]

Hyperbaric oxygen therapy is also thought to promote wound healing by suppressing growth of anaerobic bacteria.[47] Bacterial cultures from both groups taken at baseline (T1) mainly contained β-hemolytic streptococci and *S aureus*, both of which are gram-positive, aerobic species. Nevertheless, we also found β-hemolytic streptococci in both groups at baseline; this more pathogenic species was present in the routine care group at T3 but was not found in patients treated with adjunctive HBOT. *S aureus* levels in

TABLE 2.
Effects of HBOT on Inflammation Indices, Glycemic Control, and Survival Rate of Tissue in the Affected Limb by Blood Flow Perfusion Scan[a]

	HBOT Group (n = 20)	Routine Care Group (n = 18)	*P*[b]
ESR			
T1	85.7 ± 26.3	77.7 ± 29.8	.141
T2	75.4 ± 33.0	89.5 ± 32.7	.186
T3	61.8 ± 27.8	79.4 ± 29.6	.085
T4	35.5 ± 16.9	79.3 ± 33.4	<.001[c]
CRP			
T1	73.0 ± 64.7	100.2 ± 67.9	.206
T2	28.18 ± 19.5	60.3 ± 49.9	.363
T3	23.5 ± 16.1	44.1 ± 31.4	.209
T4	6.9 ± 6.6	59.0 ± 40.8	<.001[c]
HbA_{1c}			
T1	8.8 ± 2.0	8.3 ± 2.2	.534
T3	7.5 ± 1.5	7.8 ± 1.8	.228
T4	6.7 ± 1.2	7.7 ± 1.5	.002[c]
Survival rate of tissue in the affected limb			
T1	0.09 ± 0.07	0.05 ± 0.05	.091
P[d] (T1 vs T2)	.052	.285	
T2	0.16 ± 0.13	0.06 ± 0.05	.003
P[d] (T2 vs T3)	.051	.811	
T3	0.21 ± 0.07	0.07 ± 0.07	<.0001[c]
P[d] (T3 vs T4)	.183	.556	
T4	0.17 ± 0.16	0.07 ± 0.07	.026
P[d] (T1 vs T4)	.001	.476	

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; HbA_{1c}, glycated hemoglobin; HBOT, hyperbaric oxygen therapy.
[a]Data are shown as mean values ± standard deviation.
[b]Examined by the Mann-Whitney *U* test.
[c]T1, pretreatment (before the first administration of HBOT); T2, during treatment (at the 10th administration of HBOT); T3, posttreatment (at the 20th administration of HBOT); T4, treatment follow-up (2 weeks after the last therapy ended).
[d]Examined by the Kruskal-Wallis *H* test.

the wound can be used to differentiate grades of DFU, and its eradication has been associated with wound healing.[48] Two weeks following treatment (T4), we found decreased colony counts of *S aureus* in patients treated with HBOT. In contrast, control group subjects had increased colony counts of *S aureus*. We hypothesize that HBOT suppressed the growth of anaerobic bacteria and possibly impaired activation of bacterial endotoxins. It has previously been shown that HBOT provides phagocytic leukocytes with 15 times more oxygen than required when digesting microorganisms, resulting in large amounts of free oxygen radicals that kill bacteria.[49] Our findings are consistent with these results, indicating an antimicrobial function of HBOT.[1,17,50]

Treatment significantly improved HRQOL assessed by the SF-36v2 ($P < .01$). Improvements in HRQOL may be attributed to progress toward ulcer healing and decreased emotional stress. The results of this study are consistent with those of Lin's group[51] and Löndahl's group,[13] who also reported improvement in quality of life following HBOT for treatment of DFUs.

Limitations

Several elements of the design may limit generalizability of findings. Subjects were enrolled for a single medical center, and follow-up did not continue until the DFU closed. Further investigations including multiple settings may be needed to confirm the findings in this study.

CONCLUSION

Findings from our study suggest that HBOT promoted DFU healing by increasing oxygen dispersion to damaged tissues, alleviating inflammation, and suppressing the growth of anaerobic bacteria. In addition, we found that HBOT reduced the risk of amputation of the affected limb and improved HRQOL. We recommend administering at least 20 treatments to maximize the beneficial effects of HBOT on patients with nonhealing DFU.

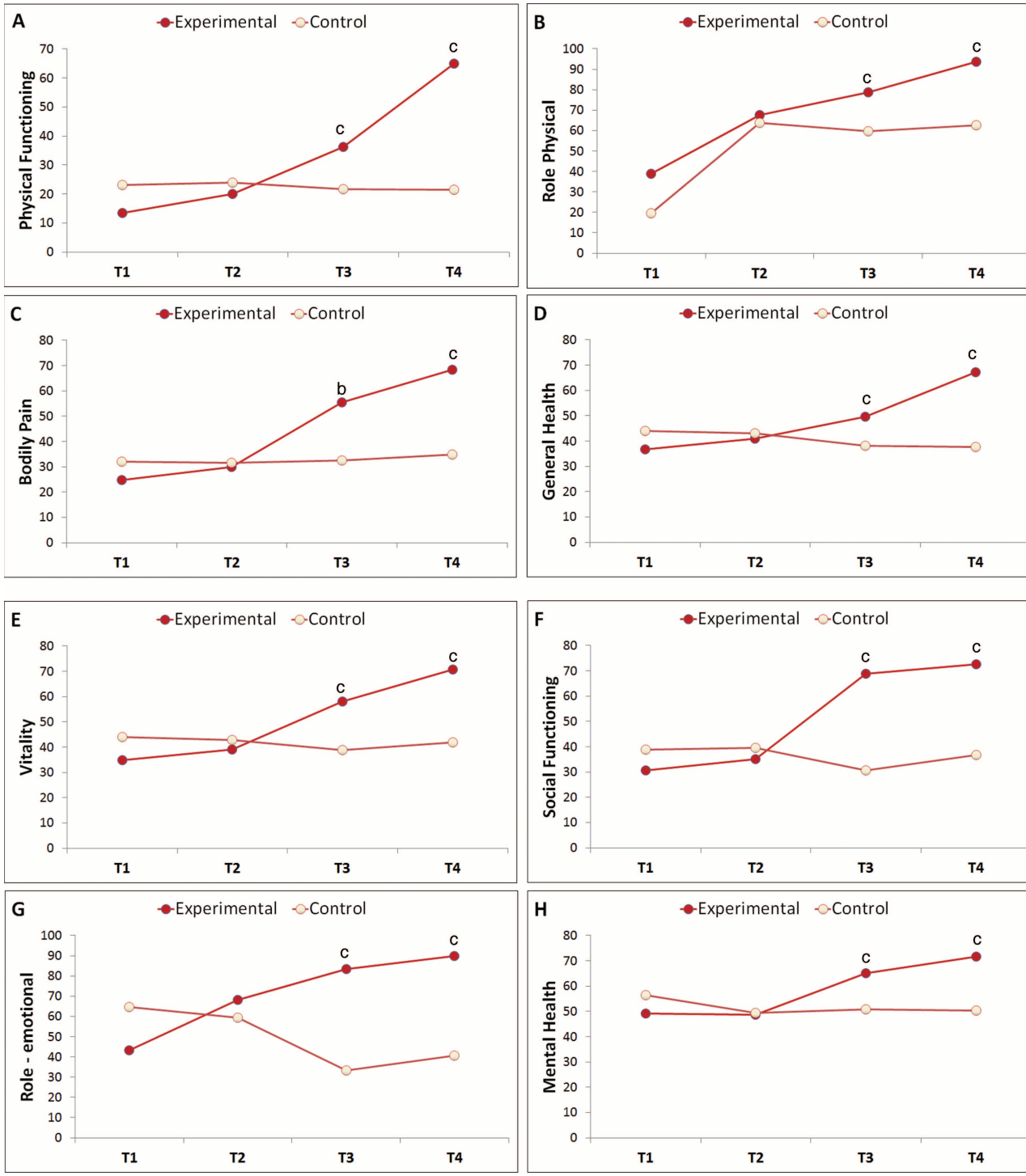

Figure 3. Effect of HBOT on Physical Component Summary score on physical functioning (A), role physical (B), bodily pain (C), and general health (D). Effect of HBOT on Mental Component Summary score on vitality (E), social functioning (F), role emotional (G), and mental health (H). [a]$P < .05$; [b]$P \leq .01$; [c]$P \leq .001$. Analysis revealed significant improvements in the physical functioning in the HBOT group at T3 and T4 compared to the routine care group (A). Both groups showed improvement in the role limitation, but significant differences between the 2 groups appeared at T3 and T4 (B). Bodily pain scores in the HBOT group began to increase at T3 and further at T4, whereas those for the routine care group did not significantly change at any point during the study. The differences between the 2 groups at T3 and T4 were statistically significant (C). The general health significantly improved in patients managed with complementary HBOT at T3 and T4 as compared to scores in the routine care group (D). Vitality scores improved at T3 and at T4 (E). Social functioning, role limitations (mental) (G) and general mental health (H) improved significantly at T3 and T4 (F). —●—, HBOT group; …o…, routine care group.

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