



RESEARCH ARTICLE

EFFECTIVENESS OF HYPERBARIC OXYGEN THERAPY ON FIBROBLAST PROLIFERATION FOR THE MANAGEMENT OF DIABETIC FOOT

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ABSTRACT

Background: Diabetic foot, an entity with great polymorphism, includes from minimal to catastrophic injuries like gangrene. Treatment is debridement, infection management, and revascularization procedures when indicated. Hyperbaric Oxygen Therapy improves tissue hypoxia and promotes fibroblast proliferation. **Goals:** To determine the effectiveness of adjuvant hyperbaric oxygen therapy as a treatment that increase fibroblasts production in diabetic foot wounds. **Methods:** Randomized double blind clinical trial; in patients with Diabetic Foot Wagner II-IV category WHO, attended at the Hyperbaric and Undersea medical Service of the Naval Hospital of Specialties in Veracruz. Inclusion criteria were ages from 40 to 60 years, and with Central glucose levels <140 mg / dL. Two groups were studied: one with conventional treatment and another with conventional treatment plus hyperbaric oxygen therapy. Fibroblast proliferation was measured by means of 2 incisional wound biopsies, taken before and after treatment. The variables used were: occupation, education, age and sex. The statistical analysis was descriptive and inferential, with T student for independent and related samples, and with P <0.05 to test hypotheses. **Results:** 50 patients were evaluated. Average age was 59.6 ± 7.2 years. 28 patients were men (56%) and 22 women (44%). The intervention group consisted of 23 patients and it was possible to demonstrate an increase in fibroblasts as represented by a count of 305.652 / mm³, while the Control group was formed by 27 patients and with a count of 73.333 / mm³. The use of hyperbaric oxygen therapy as an adjunctive treatment showed greater effectiveness for the management of diabetic foot wounds when compared with conventional treatment since the patients presented improvements in fibroblast count and proliferation, as well as in the healing of chronic ulcers.

INTRODUCTION

Diabetic foot (DF) includes a set of syndromes in which the presence of neuropathy, ischemia and infection originate in tissue or ulcers injury as a result of minor trauma, with substantial morbidity that may even end in amputation (Andersen, 2007; Sharon, 2008) The vast majority of patients have peripheral artery disease that generates increased ischemia and infection. The main microvascular complication is neuropathy that causes loss of sensation in the foot, deformities, abnormal pressure, wounds and ulcers (Sharon, 2008).

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DF ulcers can be prevented with appropriate strategies that include screening, classification of risk and treatment (Martinez, 2017). This condition is a common consultation in the services Hyperbaric Chamber and Clinic wounds, General Surgery, Emergency, outpatient, General Medicine and Family Medicine since due to the anxiety and worry that this causes to the patient with DF on not seeing improvement in healing they end up consulting at these medical services for evaluation of their ailment (Castro, 2009; O'Reilly, 2011). In these situations often the main treatment offered due to the rapid progression of complications combined poor metabolic control and high risk of fatality, this constitutes the radical treatment (minor or major amputation) (Lipsky, 2006; Subbotina, 2002; Strauss, 2005), which in many cases is unnecessary and leads to a

health problem that occupies resources and materials that can be used in case of real emergency treatment or clinical trial (Guo, 2003; Millan, 2006). The objective of this research is to propose the use of hyperbaric oxygen therapy as an adjunct to conventional treatment for DF (Hyperbaric Oxygen Broussard, 2011; Lindell, 2014; Wilbur, 2010), since several studies and clinical tests have evaluated its effectiveness in promoting the healing process by promoting fibroblast proliferation (Acosta, 2006; Arenas, 2017; Garcia, 2007), increased bactericidal capacity due to the direct toxic effect of neutrophil on certain microorganisms (Subbotina, 2006; Moon, 2010) arteriolar vasoconstriction (El-Wa'ili, 2006) with a consequent reduction of edema and ischemia-reperfusion injury (Viadé, 2013; Wattel, 1991); resulting in increased environmental pressure and hyperoxygenating of tissues in the body (Millan, 2004) In our country, we do not have current studies on prevalence or incidence of DF and hyperbaric oxygen therapy (HBOT) (Chuc, 2008; Lambrinos, 2017; Wu, 2018), studies exist on the adjuvant use of this therapy, observational indication, descriptive, retrospective and more than 5 years of decline, in which the observed effects of hyperbaric oxygen on the healing process and effect on fibroblasts are not very specific or clarified. In Mexico, the condition that registered the most deaths was Diabetes Mellitus, according to INEGI, during 2015: 98,521 people died from this disease, which caused the federal government to issue an epidemiological emergency declaration for a non-infectious disease for the first time (Ensanut, 2016)

In the ENSANUT MC 2016 the prevalence of previous medical diagnosis of diabetes in adults was 9.4%, being slightly higher in women and in urban locations. 87.8% of diabetics are under medical treatment to control their disease. In the last year 15.2% of diabetics had measurement of glycosylated hemoglobin, 4.7% of microalbuminuria, and 20.9% review of feet. The most frequent complication among people with diabetes was decreased vision (54.46%), followed by burning, pain or loss of sensation in the soles of the feet (41.17%). 46.4% of diabetics do not carry out preventive measures to avoid or delay any complication due to the disease (Ensanut, 2016)

METHODOLOGY

This was a Double-blind randomized clinical trial in patients diagnosed with Diabetic Foot II-IV category Wagner, who attended outpatient clinic Hyperbaric Medicine and Wound during the period from 15 May 2017 to 30 September 2019. Patients of both sexes were included with an age range of 40 to 70 years old, diagnosed with Type 2 Diabetes Mellitus, with adherence to treatment of underlying pathology, and who achieved the pre stipulated glycemic control goal (glucose <140 mg / dl). We excluded patients who did not attend follow-up appointments, none adherent to the treatment, or did not achieve glucose goals and also eliminated patients who did not complete the sessions of hyperbaric chamber or underwent radical treatment. The variables of the study were occupation, education, age and sex. Once this study was approved by the ethics committee and research of the Naval Hospital Specialties Veracruz, with registration number 057/18. Patients were recruited at the Outpatient Hyperbaric Medicine and Undersea and Clinical Wound, explained in clear language the benefits of the study and the expected effects of adjuvant treatment with hyperbaric oxygen therapy and through an informed consent were invited to participate in this study.

Once accepted and signed, data collection, clinical assessment and metabolic control was initiated. The patients were seen at the outpatient Wound Clinic 2 times per week for advanced wound management, and indications given not to suspend established medical treatment for the underlying disease and open appointment was given to the emergency department in case of any adverse eventualities. A randomized clinical trial with an experimental group receiving conventional treatment and OHB and a control group which was given conventional treatment was designed. The assignment to the study groups was performed by means of a lottery by the hyperbaric chamber operator (diver) and blinded to the investigator who valued response to treatment and patients attending sessions of hyperbaric oxygen therapy. He proceeded to explain to the patient and the family member responsible in a verbal and illustrative manner how to perform wound care and dressing at home, and the expected effects of OHB. Appointments were given from Monday to Friday over a period of 6 weeks for OHB sessions with advanced medical assessment and healing every week to evaluate the effectiveness of treatments in the diabetic foot wounds. Incisional biopsy was performed (removal of a small portion of tissue 1 cm thick encompassing edge and bottom of the wound) once signed informed consent prior to the start of the study, and 15 sessions of OHB, were obtained. Histopathological samples were sent for interpretation by the Pathology service of the hospital where the fibroblasts count was obtained by staining the sample with hematoxylin-eosin and using a Neubauer hemocytometer. Follow up and patient monitoring concluded in September 2019.

The results of the variables were coded in Excel 2013 for analysis in a statistical package SPSSv24.0. A descriptive statistical analysis was performed with calculation of central tendency measures (mean and median) for quantitative variable and absolute and relative values for qualitative variables. Statistical inference were made by Chi square test with Yates correction plus odds ratio (OR) and confidence interval at 95%, with statistical significance value of $p < 0.05$.

This research is based on the Good Clinical Practice with the recommendations of the Good Practices clinics Organization Pan American Health Organization, the Helsinki Declaration latest version, Fortaleza 2013, attached to the General Health Law and Regulation in the Field of Investigation. Data protection is regulated according to that suggested by the Federal Institute of Access to information in the document "Report on access to clinical records" of 2004. At point "7.2. Data processing for research purposes "where usage is mentioned without this undermining the confidentiality of the patient. Health Standards in Research for Health in humans, stipulates that ethical aspects should be established to ensure the dignity and welfare of the person under investigation; should employ techniques and research methods documentary retrospective and those in which no intervention or intentional modification of physiological, psychological and social variables of individuals participating in the study, including those considered not performed: questionnaires, interviews, review of clinical and other records, which are not identifiable or sensitive aspects are dealt with their behaviour. The main objective of this study is to protect life, health, dignity, integrity, right to self-determination, privacy and confidentiality of personal information from people involved in research and properly informed of the objective and the form of data collection in the study, report any risks that could be presented by participating.

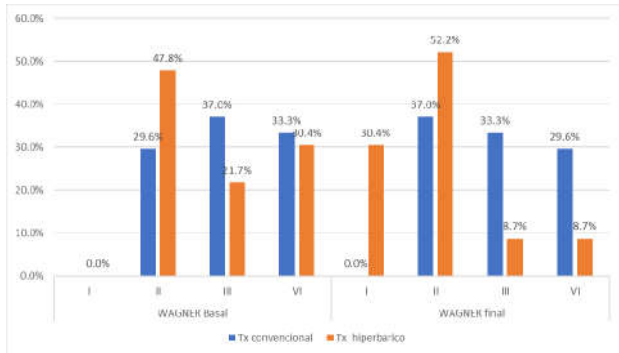


Figure 1. Type distribution wound by Wagner scale according treatment

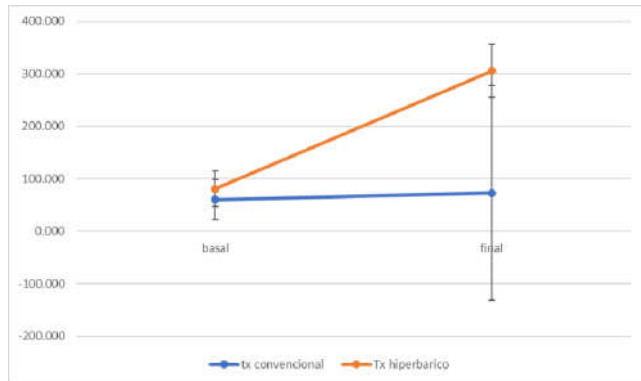


Figure 2. Fibroblast proliferation by type of treatment

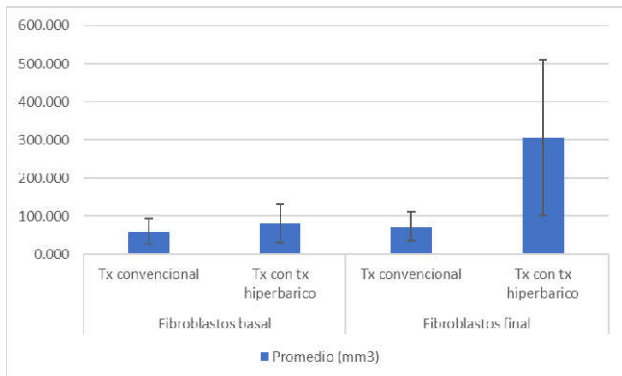


Figure 3. Group distribution study of fibroblast proliferation

Table 2. Wound classification scale by Wagner as treatment

Wagner classification	Group A Conventional treatment + OHB n = 23	B Group conventional treatment n = 27
At the start of the study		
grade I	0 (0%)	0 (0%)
grade II	8 (29.6%)	eleven (47.8%)
grade III	10 (37.0%)	5 (21.7%)
grade IV	9 (33.3%)	7 (30.4%)
grade V	0 (0%)	0 (0%)
At the end of the study		
grade I	0 0.0%	7 30.4%
grade II	10 37.0%	12 52.2%
grade III	9 33.3%	two 8.7%
grade IV	8 29.6%	two 8.7%
grade V	0 0.0%	0 0.0%

This type thesis research was carried out with the prior approval of the study subjects or their representatives legal as well as the HOSNAVESVER; after signing the informed consent of the participants of the study and approval of the project by the Research Ethics Committee of the HOSNAVESVER, book registration number 057/18. The participation on study subjects was done freely and autonomously. Participants were informed about the research and selected fairly and equitably, without personal bias or preference. The research participants maintained their protected privacy, having the option to leave the study if necessary. Management information obtained from clinical records was strictly confidential and not misused.

RESULTS

In the HOSNAVESVER, during the period 15 May 2017 to 31 January 2019. The study included 50 patients with diabetic foot that met the selection requirements of the study, without exclusions or limitations. 50 patients were included in the study. They were 28 men (56.0%) and 22 women (44.0%). With an age range of 40-70 years and randomized into two treatment groups: Experimental group (A), conventional treatment + hyperbaric oxygen (23 patients) and control group (B) conventional treatment (27 patients). Mean age was similar in the two groups, with an average age of 59.6 years with standard deviation ± 7.2 years, with p value of 0.08. The age of group A was 61.8 (SD ± 7.3) years and those of the B 57.8 (SD ± 6.7) group. The sex distribution showed a higher frequency for males in group A (56.5%) and group B (55.6%). Education level ranged from 6to. Primary (73.9%) and group A (74.1%) Group B.

There is no statistically significant association between sex or education and severity of diabetic foot wounds. (Table 1). Ranking diabetic foot wounds by Wagner scale, at the beginning of the study, 0 patients were found to be Grade I, 19 patients (38.0%) Grade II, 15 patients (30.0%) Grade III, 16 patients (32.0%) were grade IV and 0 patients grade V. 0. Obtaining at the end of the study 7 patients 14.0% grade I, 22 patients 44.0% grade II, 11 patients 22.0% grade III, 10 patients 20.0% grade IV grade and 0% V. (Table 02 and Figure 01). Patients included in the study showed on final assessment predominance of fibroblast proliferation in group A conventional treatment + hyperbaric oxygen therapy, with the analysis showing statistically significant for the different groups. The same results were found comparing the medians of the two groups and on contrasting the improvement at least

Table 1. Comparing age, sex, occupation and educational level as type of treatment of diabetic foot

	Group A Conventional treatment + OHB n = 23	B Group conventional treatment n = 27	Value of p
Age (years of life)	61.8 ± 7.3	57.8 ± 6.7	0.08
Sex			
Male	56.5%	55.6%	0.9
Female	44.4%	43.5%	
Occupation			
active military	0 (0%)	1 (4.3%)	0.3
Retired military	8 (29.6%)	4 (17.4%)	
rightholder	19 (70.4%)	18 (78.3%)	
Scholarship			
Primary	20 (74.1%)	17 (73.9%)	0.8
primary incomplete	1 (3.7%)	2 (8.7%)	
High school	3 (11.1%)	3 (13%)	
secondary	1 (3.7%)	0 (0%)	
incomplete	1 (3.7%)	1 (4.3%)	
career technical	0 (0%)	0 (0%)	
Bachelor's degree	1 (3.7%)	0 (0%)	
illiterate			

two degrees in the classification of Wagner. In addition, it was observed that while patients with conventional treatment only presented chronicity of wounds ($p > 0.05$); neither groups presented cases of infection related with the application of drugs or hyperbaric oxygen therapy. In comparing hyperbaric clinical efficacy of conventional treatment plus oxygen versus the control group with conventional treatment and considering the lack of improvement as event outcome it was found that the absolute risk of the control group was 80.0% compared to 5.0% of the intervention group allowing the acquiring of a relative risk (RR) of 0.06, 95% (.01-.43), the relative risk reduction (RRR) of -93.8%, 95% (-99.1% -57.2% a), reducing the risk absolute (RRA) of -75.0%, 95% (-95.0% -55.0% to) and a number needed to treat (NNT) of 1, 95% (1 to 2).

DISCUSSION

The findings of the present study show that the efficacy of adjuvant treatment with hyperbaric oxygen therapy for the wound management of diabetic foot achieved significant improvement in terms of fibroblast proliferation and activation of the process of wound healing of diabetic foot, whilst the application of conventional treatment presented minor improvement. Likewise the time required to observe a favorable response to treatment and glycemic control was significantly lower with hyperbaric oxygen therapy plus conventional therapy and safety of the treatment was possible due to the fact that no case with adverse effects occurred. The results of this study are relevant if we consider that they come from a robust design to evaluate the efficacy of a treatment, establishing computerized procedures randomization, blinding and complete patient follow-up, however, a weakness could be considered for not having a hard measurement beyond the visual evaluation of fibroblast proliferation incisional wound biopsy provides. The efficacy demonstrated in our work by the administration of adjuvant treatment with hyperbaric oxygen therapy for wound management diabetic foot is compatible with that found in previous studies, however in regards to the fibroblast proliferation effect of hyperbaric oxygen therapy observed in our study, this was not considered in any of the previous studies.

Our findings highlight that adjuvant treatment with hyperbaric oxygen therapy is a treatment option of high efficiency and safety for wound management of diabetic foot that can be prescribed in primary care or outpatient Wounds clinic and contribute to the reduction of the frequency of this disease which is highly prevalent in people with Diabetes Mellitus and its potential complications. With regard to the safety of the use of hyperbaric oxygen therapy, the data obtained in this study confirm the virtual absence of adverse effects to the treatment, as reported in the literature shown in studies in Spain, USA, Canada, UK and other countries. To summarize current evidence shows that the risk of adverse effects is not significant in dealing with adjuvant hyperbaric oxygen therapy, taking into account the management for hyperbaric oxygen therapy should be undertaken by expert hands, meaning Doctors specialists in Hyperbaric Medicine and Sub aquatic, and not by unqualified personnel. In our study, the mean age of patients with diabetic foot and the time evolution of Diabetes Mellitus since his diagnosis coincides with the published series of patients who consult for diabetic foot complicated. One aspect to discard is that almost 10% of patients, when consulted, had less than 3 years from diagnosis of Diabetes Mellitus, so it is in line with the silent course of its main responsible factor, neuropathy and poor glycemic control.

Psychosocial aspects such as the negative profile that envelops these patients could promote that they do not benefit from early diagnosis and consult in situations more advanced disease. This aspect could also contribute to the poorer glycemic control and higher frequency of complications in terms of diabetic foot wounds. Globally, studies have shown that reducing the risk of amputations in patients with DM and the use of hyperbaric oxygen therapy, reflects a better metabolic control, as well as a systematic and routine review of the feet, focus should be placed on efforts in educating the patient and family on the care of Diabetic Foot.

Conclusion

The application of hyperbaric oxygen therapy plus conventional treatment in diabetics with torpid wounds constitutes a therapeutic intervention of high efficacy and safety for the management of diabetic foot, superior to treatment with cures, wound debridement, without presenting side effects, so its administration is recommended Timely as soon as the diagnosis is established. Contrary to what many people think, adding hyperbaric oxygen to conventional diabetic foot treatment, in addition to lowering amputation rates, is cheaper than standard treatment by decreasing the days of hospital stay, medications, use of operating rooms; etc. With respect to the availability of a camera in Mexico there are more than 150 cameras installed and functioning. Diabetic foot treatment is a chain of scientifically approved tools, if one of them is missing the result will be bad. Hyperbaric oxygenation can be considered another link in that chain. This technology is not "the cure of everything" or the panacea, and the inappropriate use that some doctors make of the hyperbaric chamber is their responsibility only, behavior that should always be pointed out negatively, but that should not be a cause of attack on the benefits of this technology.

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