ROLE OF HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF DIABETIC FOOT

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ABSTRACT

This single blind study was conducted over two and half years period of 30 diabetic patients with foot problems to asses the effect of hyperbaric oxygen therapy (HBO). They were divided into study and control groups. The two groups were age and sex matched and divided in a randomised fashion.

The control group was subjected to 2.8 ATA pressure whilst breathing 7.5% oxygen and 92.5% nitrogen. The study group was subjected to 2.8 ATA partial pressure compression utilizing 100% oxygen. Both the groups received regular surgical treatment, antibiotics and insulin (most often multiple subcutaneous insulin [MSI] regime.)

The overall outcome was better in the study group with 86.6% of patients showing complete healing or marked improvement as compared to 46.7% in 0controls ($P < 0.01$). The study group received significantly less number of HBO sittings as compared to the controls. Grades 1,2,3 feet fared better with 92.3% of the study group having benefitted. Maximally benefitted were patients with mixed and neuropathic ulcers.

The most striking effect of HBO therapy was seen in infection control. 78.9% of the controls had positive wound cultures post-HBO compared to only 10% in the study group which is significantly less($P<0.001$)The study group required only 12.2± 4.1 HBO sittings compared to 20.4± 6.9 in controls to achieve culture sterility. Non smokers in the study group did significantly better (91.7%) as compared to controls (55.5%) ($p < 0.05$).

In the control group none required minor amputation whilst one patient in the study group required the same. This reflects the ability of HBO to limit spread of the disease and keep the level of amputation low. Two patients in the control group required major amputation s compared to one in the study group. There was a significant ($P < 0.001$) fall in the blood glucose levels post HBO in both groups. However, the insulin like role of HBO is yet to be established.

In this study no patient experienced any major complications of HBOT. There was no increase in cardio-pulmonary or central nervous system related complications. Claustrophobia was experienced by three patients in the study group and four patients in the control group, but this did not necessitate discontinuation of therapy.

HBO is thus a useful adjunct in the overall care of a diabetic with foot problems. It is also found to control progression of disease and to reduce the morbidity.

***KEY WORDS* :** Diabetic foot, hyperbaric oxygen therapy.

INTRODUCTION

Diabetes mellitus (DM) is accompanied by long term microvascular, neurologic and macrovascular complications. Although the daily management of DM is burdensome and the specter of metabolic decompensation ever present, long term complications including retinopathy, nephropathy, neuropathy, diabetic foot and cardiovascular disease, have caused significant morbidity and mortality since the introduction of insulin therapy, consequent to the increased longevity in diabetics. The prevention and amellioration of these complications have been the major goals of recent research [1].

Available data has shown that HBO therapy has great potential for accelerated healing, reduction in number and size of amputations, reduction in duration of hospital stay and control of infection of the diabetic foot. The paucity of literature and the beneficial effect of this relatively harmless therapy on the favorable outcome of this dreaded complication of diabetes, prompted the conduct of this study.

MATERIALS AND METHODS

Material

The study was conducted in age matched groups in a randomised fashion. For the purpose of this study a total of 30 patients were included during a period of two and a half years. The study group consisted of 13 males and two females ($n = 15$). The total number of patients in the control group was 15, including three females and 12 males.

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The study was conducted by a combined team of the Department of Endocrinology, INHS Asvini and the Center for Hyperbaric Medicine, Institute of Naval Medicine (INM), INHS Asvini. The study group received HBO therapy as scheduled. The control group was subjected to the same therapy nd pressures as the study group, in the recompression chamber but a 7.5% oxygen mixture was employed. This ensured a partial pressure of 0.21 ATA (atmosphere) in the recompression chamber, the same as the partial pressure of oxygen in the ambient atmosphere.

The patients were thoroughly examined to evaluate their fitness to undergo HBO therapy. The pre HBO therapy investigative armamentarium included detailed clinical examination, complete ENT examination, chest roentgenogram, ECG, blood glucose profile, hemoglobin oxygen saturation (pulse oximetry), wound swab culture and antibiotic sensitivity testing of the micro organism.

The two groups were age matched and divided in a randomised fashion. To minimise bias, the referring clinician was blinded while the physician conducting HBO therapy knew the composition of each patients's breathing gas during HBO therapy.

Upon referral, all patients underwent an extensive consultation period to determine if they complied with the rigid acceptance standards. During this evaluation period, each patient was made aware of the design, execution and possible outcome of the therapy. Participation was strictly voluntary. The study was approved by the ethical committee of INHS Asvini. The mechanism of action and the expected benefits as well as the anticipated side effects of HBO therapy were explained to each patient in a language and terminology understandable to them and a written consent obtained. All patients were handled uniformly by providing daily wound care (if required); each patient entered the chamber and particiated in each treatment dive, each patient was decompressed safely to surface with a 20 minute linear ascent.

ACCEPTANCE STANDARDS

1. Patients with stable vital parameters and stable haemodynamic parameters.

2. Mobile tympanic membranes on ENT examination (Valsava manouvre). In those patients with negative Valsalva manouvre due to non-patent eustachian tube (either unilateral or bilateral) due to upper respiratory tract infections or due to any other cause, myringotomy was performed prior to therapy.

3. Patients who had no roentgenographic evidence of pneumothorax, pleural effusion, emphysema, bronchiectasis, cavities, fibrosis or any other pathological causes for air trapping in the lungs. Patients with X-ray evidence of cardiomyopathies or pericardial effusions were not included in the study.

4. Patients with gross ECG abnormalities and conduction disturbances were excluded.

5. Patients who had major macroangiopathic involvement of major arteries proximal to the lesion were excluded.

CLASSIFICATION OF THE LESION :

The lesions were classified using Wagner's classification. Grade O foot has no open lesions, but is the 'at risk' foot. Grade 1 lesions consist of a superficial ulcer, but with full thicknes skin loss. Grade 2 lesion is deep and often penetrates subcutaneous fat down to tendon or ligaments, but without abscess formation or bony infection. Grade 3 lesions have deep infection with cellulitis or abscess formation, often with underlying osteomyelitis. The main difference from grade 1 and 2 foot is that in grade 3 foot, surgery is usually needed. The hallmark of grade 4 foot, is gangrene, which may be localised to a toe, a small area of the heel or involve more of the distal foot. Grade 5 lesions are characterised by extensive gangrene of the foot and need urgent hospital admission, control of diabetes and infection and usually a major amputation.

Methods :

The facilities available in the Hyperbaric Centre, School of Naval Medicine, INM, INHS Asvini, includes a monoplace recompression chamber (Roberto Galleazi, Spa, Italy) and a multiplace recompression chamber (COMBEX, France), with associated equipment panels, BIBS (built in breathing systems), Bauer air and oxygen compressors and mixture gas banks. These were utilised for the conduct of this study. The chambers are equipped with viewing portholes and communication systems (with unscramblers) for patient monitoring.

All patients in the study and control group received regular surgical treatment consisting of incision and drainage of abscesses and wound debridement Antibiotics were administered along with metronidazole. Antibiotics commonly used were cephalosporoins and aminoglycosides and were

changed according to antibiotic sensitivity patterns Diabetic control was achieved by insulin, most often using MSI regime.

CONTROL GROUP :

15 patients made up the control group. Average age was 67 ± 9.81 years (range 45-85 years). These patients were subjected to 2.8 ATA pressure in the Multiplace/Monoplace recompression compression chamber. The breathing mixture was made of 7.5% oxygen and 92.5% nitrogen which at 2.8 ATA ensured an oxygen partial pressure, in the breathing mixture, of 0.21 ATA (equivalent to the oxygen partial pressure of air at sea level).

STUDY GROUP:

The study group consisted of 15 patients. The average age of the study group was 63.1 ± 8.74 years (range 51 – 75 years). These patients were subjected to 2.8 ATA partial pressure of oxygen by compressing them in the Mono/Multiplace chamber, breathing gas being 100% oxygen.

Thus the control group received the same percentage of oxygen as at ambient atmospheric level, while the study group was exposed to 100% oxygen or truly hyperbaric oxygen therapy.

HBO SCHEDULE :

All patients (control and study group) were subjected to undergo a therapeutic dive in either the monoplace or multiplace recompression chamber, for one hour bottom time (stay at the desired pressure) once a day, at 2.8 ATA (18 meters). The decompression to surface was a 20 minute linear ascent. In addition to HBO, all patients received routine surgical care. If after ten HBO sittings the patient in the study group showed no improvement the procedure was abandoned.

Lesions were termed as healed when there was full dermal epithelialisation of the wound, there was no discharge and no underlying osteomyelitis. They were termed as improved when there was presence of healthy granulation tissue formation, absence of purulent discharge and pus culture was sterile. However, dermal epithelialisation was not complete. Lesions which showed negligible or no response after a minimum of ten sittings were termed as no effect.

RESULTS:

There were a total of 30 patients, equally divided in the study and control groups. Mean age of study group was 63.1 ± 8.7 years and was 67 ± 9.8 years in the controls. Response to HBOT in the respective groups is shown in Table 1. Whereas eight patients in control group had no significant change following therapy, only two in study group did not respond. This difference was significant ($P < 0.01$).

Table 1 : Response to HBOT

	Control Group n = 15	Study Group n = 15	X^2	P
Healed	5 (33.3 %)	10 (66.6 %)	3.33	NS
Improved	2 (13.4 %)	3 (20 %)	0.25	NS
No effect	8 (53.3%)	2 (13.4 %)	5.4	0.01*

When the foot lesions were graded, two each from the study and control group had Wagner's grade 1 diabetic foot, nine each had Grade 2 foot, two each had Grade 3 foot and one each had Grade 4 and Grade 5 foot problems respectively. The response to therapy as per grading of the foot is shown in Table 2. Significant benefit of HBOT was observed in grade 1,2 and 3 feet as 76.9% in study group showed complete healing compared to 38.5% in the control group and failure of therapy was observed in only 7.7% of study group compared to 46.1% in the control group. In Grades 4 and 5 feet, one patient (50%) showed improvement following HBOT, whereas there was no response in the control group.

Table 2: Comparision of grading and response in both groups.

	Control			Study		
	No	%	X2	No	%	P
Grade	1,2,3	(n = 13)			(n=13)	
Healed	5	38.5	3.94	10	76.9	<0.05*
Improved	2	15.4	0	2	15.4	>0.05**
No effect	6	46.1	12	1	7.7	<0.001***
Grade	4,5	(n = 2)			(n=2)	
Improved	_	_		1	50	
No effect	2	100		1	50	

* significant, ** not significant, *** highly significant

Another remarkable feature was that an average of 29 ± 4.9 sittings were required for healing in control (placebo) group compared to 19.1 ± 4.7 sitting in the study group. Similarly in those patients who improved, the number of sittings in control versus study group were 42 ± 7.1, compared to 26.3 ± 6.1, and both these findings were statistically significant (Table 3).

Table 3: Number of HBOT sittings required to reach Healed or Improved state

	Control	Study	t
Healed	29 ± 4.9	19.1 ± 4.7	5.56*
Improved	42.2 ± 7.1	26.3 ± 6.1	6.57*

P < 0.001 (highly significant)

Culture positivity before and after HBOT in control and study groups is shown in Table 4. Whereas 78.9% remained culture positive following HBOT in the control group, only 10% patients had positive cultures in the study group. This difference was satistically significant (Table 4). Similarly in those patients who had complete culture sterility, the number of sittings required in the control group was 20.4 ± 6.9 compared 12.2 ± 4.1 in the study group (p < 0.0001) (Table 5).

Table 4 : Microbiological studies.

	Control		Study	
	Before HBOT(%)	After HBOT(%)	Before HBOT(%)	After HBOT(%)
Pseudomonas	6 (31.6)	8 (53.3)	5 (25)	1 (50)
E. Coli	5 (26.3)	5 (33.3)	7 (35)	1 (50)
Staphylococci	3 (15.8)	0	4 (20)	0
Klebsiella	3 (15.8)	2 (13.4)	1(5)	0
Bacteroides	1 (5.2)	0	1 (10)	0
Peptostreptococcus	1 (5.2)	0	2 (10)	0
Total	19	15 (78.9)	20	2 (10)*

p < 0.001 (Significant)

Table 5 : Mean number of sittings to obtain culture sterility

Control	Study	t value	Significance
20.4 ± 6.9	12.2 ± 4.1	3.96	p < 0.001

When the diabetic foot problems were subclassifed into neuropathic, vascular and mixed varieties, the best response was seen in the neuropathic foot, followed by mixed and the least in the vascular variety (Table 6) Non-smokers showed a better response

Table 6 : Etiology of foot problems and response

Control Group n = 15	**Study Group n = 15**	
Neuropathic	**4**	**6**
Healed	2 (50 %)	5 (83.3 %)
Improved	1 (25 %)	5 (16.7 %)
No effect	1 (25 %)	nil (0%)
Vascular	**2**	**2**
Healed	nil (0 %)	nil (0 %)
Improved	1 (50 %)	1 (50 %)
No effect	1 (50 %)	1 (50 %)
Mixed	**9**	**7**
Healed	2 (22.2 %)	4 (57.1 %)
Improved	1 (11.1 %)	1 (14.3 %)
No effect	6 (66.7 %)	2 (28.6 %)

In this table no statistical test was applled as the number of samples were not adequate.

than smokers in both control and study groups (Table 7). Two patients in the control group required major amputations. One required minor and another required major amputation in the study group. The mean fall of blood sugar before and after HBOT was 94.3 ± 18.2 mg/dl and 95.8 ± 17.6 mg/dl in the control and study groups respectively. This difference was not significant (Table 8).

Table 7 : Smoking and response to HBOT

	Control n = 6	**Study n = 3**
Smoker		
Healed	1 (16.6 %)	1 (33.3 %)
Improved	1 (16.6 %)	1 (33.3 %)
No effect	4 (66.8 %)	1 (33.3 %)
Non Smoker	**n = 9**	**n = 12**
Healed	4 (44.5 %)	9 (75 %)
Improved	1 (11 %)	2 (16.7 %)
No effect	4 (44.5 %)	1 (8.3 %)

Table 8 : Blood glucose response before and after HBOT

	Blood Glucose (mg/dl)	
	Control Group n = 15	**Study Group n = 15**
Before	250.4 ± 40.2	240.6 ± 32.9
After	156.1 ± 20.4 *	144.8 ± 19.7 **
Mean Fall	94.3 ± 18.2	95.8 ± 17.8 ***

*t=5.41, p < 0.001; **t=4.97, p < 0.001
*** Difference between control and study group not significant

No significant side effects of HBOT were observed either in the control or study groups. However two patients in the study group and one patient in the control group required a myringotomy prior to proceeding with HBOT. Claustrophobia, especially in the monoplace chamber, was reported by three patients in the study group and four patients in the control group. Again this did not necessitate discontinuation of therapy and the patients overcame this feeling by counselling and if required, shifting them to the multiplace chamber.

DISCUSSION :

The overall outcome was significantly better in the study group as compared to the control group. 86.6% in the study group either healed or improved. In the study group 13.4% of the cases had no effect as compared to 53.3% in the control group ($P < 0.01$). (Table 1). Smith et al noted partial or complete healing in 64% of cases (n = 36) with diabetic foot problems [2].

In the control group, the average number of HBO sittings required to achieve complete healing was 20 ± 4.9 and for improved state it was 42.2 ± 6.1. The controls therefore required significantly more ($p < 0.001$) number of HBO sittings to heal or improve as compared to the study group counterparts (Table 3). In both the study and control goups, the number of sittings required to achieve complete were fewer than those required for improvement, probably because in the latter cases, the underlying pathology was relatively more severe. Barroni G et al treated 18 diabetic patients were healed, with the number of HBO treatments required for healing being significantly related to the size of the gangrenous lesion [3].

The Wagner's grade 1 foot healed in both control and the study groups. Five of the nine patients of the grade 2 foot in the control group had 'no effect' with treatment, whilst seven of the nine cases 'healed' in the study group with one having 'no effect'. In patients with grade 3 foot, of the two cases in the study group, one healed and the other had no effect, while in the control group, one 'improved' with the other having 'no effect'. In patients with grade 4 foot, the one in the study group improved, while the one in control group had no effect. The two patients with grade 5 feet (one in control and one in study group) did not show any response to therapy. Statistical analysis of the above data reveals that only 53.9% of the total cases of Grade 1, 2, 3 in the control group either 'healed' or 'improved'. In comparison, significantly more ($p < 0.05$) (92.3%) number of cases in the study group either healed or improved For grades 4 and 5 in both the groups, no statistical analysis was applied, as the number of cases were few (Table 2). The derivation from this study is that the outcome of case of diabetic foot is related to it's grade, with grade 1 feet faring better than grade 5 feet, which has the worst prognosis. 92.3% of Grade 1, 2, 3 in the study group healed/improved whilst in the case of Grade 4 only 50% of cases improved and none with grade 5 responded.

Table 6 shows that those with mixed and neuropathic ulcers in the study group benefitted the most. Statistical analytical tests could not be applied as the number of cases were few. In the vascular type of diabetic ulcers only one patient in the study group 'improved'. In the control group two had vascular ulcers, of which one improved whilst the other one had 'no effect'. However, none of these patients had acute arterial occlusion and neither did they have any major limb amputation. In the mixed foot, of the nine patients in control group, six had no effect with therapy, whilst one 'improved' and two "healed". These findings are consistent with the known biochemical and cellular effects of HBOT.

Many non healing wounds are hypoxic and the tissue oxygen tensions are in the range of 5 – 15 mm of Hg [4]. Although hypoxia may serve as an initial stimulus to the healing process[5], tissue oxygen tensions of 30-40 mm of Hg are necessary to promote fibroblast proliferation and the formation of a collagen matrix and which can then serve as a scaffolding for the ingress of new blood vessels[5-5]. Enhancement of WBC killing ability may be an additional benefit, particularly in the foetid foot[8]. The transient vasoconstriction caused by breathing pure oxygen at pressure, may serve to reduce edema in the neuropathic foot. As oxygen dissolves in plasma according to Henry's law, an arterial PO_2 of 1100 to 1400 mm of Hg may be achieved by breathing pure oxygen at the pressure equivalent of 2 ATA [9, 10]. HBO therapy though quite useful in diabetic wounds is inappropriate if the large vessels distal to the trifurcation at the knee are occluded or severely stenotic [11].

The most striking effect of HBO therapy was seen in the control of infection. Statistical analysis of the data shows that in the control group after HBO, 78.9% had positive wound cultures as compared to only 10% in the study group, which is significantly less ($p < 0.001$).

In cases of diabetic foot ulcers, most mild infections are caused by aerobic gram positive cocci such as Staphylococcus aureus or Streptococci [12, 13]. Deeper limb infections are usually polymicrobial and are caused by aerobic gram positive cocci, grm

– negative bacilli (eg. Escherichia coil, Klebsiella species, and Proteus species) and anaerobes (Bacteroid species and Peptostreptococcus) [14]. The pathogenic role of coagulase negative Staphylococci, Enterococci and Corynebacterium species is often difficult to discern, particularly when they are cultured along with typically virulent organisms. In this study, HBO therapy effectively decreased E. Coil and Pseudomonas infections in the study group as compared to the control group. It also controlled anerobes. However, the anaerobic infection was also eradicated by local wound care and antibodies. Staphylococci were eliminated by HBOT and also in the controls.

Diabetic patients are more prone to develop infection and there is good evidence that the rate of infection parallels blood sugar levels [15]. This increased incidence is probably related to impairment of the immune responses in diabetics [16]. ± variety of defects in leucocyte function have been demonstrated including impairment of chemotaxis[17], phagocytosis[18], intracellular bactericidal activity[19] and serum opsonisation[20]. Whilst there is some evidence that these abnormalities are related to blood sugar levels[21], other workers have shown an intrinsic defect in the leucocytes independent of extrinsic environmental factors[22]. Diabetic patients appear to have normal levels of immunoglobin[23] but there is evidence of impaired cell mediated immunity[24].

Local hypoxia predisposes wound to infection, because the neutrophil-mediated killing of bacteria by free radicals is decreased[25, 26]. Hyperbaric oxygen restores this defence against infection and increases the rate of killing of some of the common bacteria by phagocytes[8]. In addition, hyperbaric oxygen is bactericidal for certain anaerobes, including Clostridium perfringens[27], and bacteriostatic for certain species of Ecsherichia[30]. It also suppresses Clostridial production of alpha toxin. It improves antibiotic efficiency, as it has been shown that antibiotic transport into bacteria is oxygen dependent.

In the control group, average number of HBO sittings required for infection to clear were 20.4 ± 6.9 in those cases in which the post HBO wound swabs were negative. This was significantly more ($p < 0.001$) as compared to the average of 12.2 ± 4.12 sittings required in the study group for infection to clear (table 5).

There were six smokers in the control group and three in the study group (Table 7). Of the six smokers four had ‘no effect’ and one ‘improved’. In the study group (n =3) one ‘healed’, one ‘improved’, whilst one had ‘no effect’. In the control non smoker group (n = 9), four ‘healed’. Statistical analysis of the above data indicates that among the non smokers in the control group only 55.5% of the total cases responded to HBO as compared to 91.7% in the study group which is significantly more ($p < 0.05$). The comparison was not done in smokers as the number of cases were not adequate. These results could be due to the effects of tobacco on the circulatory system. Caputo et al advised that patients must refrain from using tobacco to protect the arterial circulation (31). Although some of the adverse cardiovascular effects of smoking are mediated via nicotine, the principal mechanism for the adverse effect of smoking is probably via increased concentration of carboxyhaemoglobin and resultant tissue hypoxia. Carboxyhaemoglobin has been directly shown to impair hepatic metabolism of lipoprotien remnants, the resulting remnanat accumulation results in type III hyperlipidemia, a lipid disorder known to be associated with accelerated atherogenesis. Additional mechanisms possibly contributing to the atherogenic response to smoking include n increased endothelial permeability caused by carbon monoxide induced hypoxia, resulting in an increased lipid uptake as well as an increased platelet adhesiveness.

In the diabetic, smoking may play a role in the accelerated atherogenesis, since additional factors promoting tissue hypoxia, such as a shift in the oxyhaemoglobin dissociation curve to the left, are already present. These additional factors includes reduced 2:3 disphosphoglycerate, hyperlipoproteinemia, and the increased glycosylated hemoglobin (32). Smoking has a role in the number of diabetic foot patients requiring amputation (33). However, Smith et al reported no difference in the outcome of treatment of 36 insulin dependant diabetic foot ulcers, whether they smoked or not (2).

In the control group none required minor amputation whilst in the study group one patient required the same. This reflects the ability of HBO to effectively limit the spread of disease and keep the level of amputation as low as possible. Two patients in the control group required major amputations and in the study group the figure was one. Since the number of cases were few, no statistical analytical tests could be applied.

There was a significant ($P < 0.001$) fall in blood glucose levels post HBO as compared to pre HBO levels, both in the control and study groups (TBLW 8). However, the role of HBO in assisting glycemic

control is still anecdotal and remains to be established.

REFERENCES :

1. The Diabetic Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus. NEJM, 1993 Vol 329 No 14, 977-86.

2. Smith VC, Murphy BP, Cramer FS: Hyperbaric Medicine Proceedings of the eighth Internationl Congress. Ed: Eric P. Kindwall, Milwaukee, Wisconsin. Best publication. 1984 : 204-6.

3. Baroni G, Porro T, Faflia E et al. Hyperbaric oxygen in diabetic gangrene treatment. Diabetes Care 1987 Jan-Feb : 10 (1) : 81-6.

4. Shreiff PJ, Dunn JM. Continuous monitoring of tissue oxygen tension during hyperbaric oxygen therapy. In: Smith G Ed. Proceedings. Of the sixth International Congress on Hyperbaric Medicine, Aberdeen, Scotland Aberdeen University Press, 1977.

5. Knighton DR, Oredffson S, Banda M, Hunt TK. Regulation of repair, hypoxic control of macrophage mediated anfiofenesis. In: Hunt TK, Heppenstall RB, Pines E, Rovee D, Eds. Soft and hard tissue repair. New York: Prager Publishers, 1984 : 41-9.

6. Hunt TK, Pai MP. The effect of varying oxygen tensions on wound metabolism and collagen synthesis. Surg Gynaecol Obstet 1972; 135 : 561-7.

7. Ninikoski J, Hunt TK. Oxygen tensions in human wounds. J Surg Res 1972; 12 : 77-82.

8. Mader JT, Brown GL, Guckian JC, Wells CH, Reinarz JA. ± mechanism for the amelioration by hyperbaric oxygen of experimental staphylococcal osteomyelitis in rabbits. J Infect Dis 1980; 142 (6) 915-22.

9. Committee on hyperbaric oxygenation: The compressed gas atmosphere. IN: Fundamentals of hyperbaric medicine, Publication No. 1298, Library of Congress Cat. No. 65.61028. Washington, DC: National Academy of Sciences – National Research Council, 1966 : 3-11.

10. Committee on hyperbaric oxygenation: The physiological basis of hyperbaric therapy. In: Fundamentals of hyperbaric medicine, Publication No. 1298, Library of Congress Cat. No. 65.61928. Washington, DC. National Academy of Sciences – National research Council, 1966 : 33-5.

11. Kindwall E: British Medical Journal. Vol. 307, 28 Aug 1993 : 515-6.

12. Jones EW, Edwards R, Finch R et al. ± microbiologic study of diabetic foot lesions. Diabet Med 1984; 2: 213-5.

13. Lipsky BA, Pecoraro RE, Wheat LJ. The diabetic foot: Soft tissue and infection. Infect Dis Clin North America 1990; 4 : 409-32.

14. Wheat LJ, Allen SD, Henry M et al. Diabetic foot infection: bacterial analysis. Arch Intern Med 1986; 146: 1935-40.

15. Rayfield EJ, Ault MJ, Keush GT, Brothers MI, Nechemias C, Smith H. Infection and diabetes: the case for glucose control. ± m J Med 1982; 72: 439-50.

16. Robertson HD, Polk HC. The mechanism of infection in patients with diabetes mellitus: a review of leucocyte malfunction. Surgery 1974; 74: 123-8.

17. Mowat AG, Baum J. Chemotaxis of polymorphonuclear leucocytes from patients with diabetes mellitus. New Engl J Med 1971; 284 : 621-7.

18. Bagdade JD, Root RK, Bulger RJ. Impaired leucocyte function in patients with poorly controlled diabetes. Diabetes 1974;23:9-15.

19. Nolan CN, Beaty HN, Bagdade JD. Further characteristics of the impaired bacterial function of granulocytes in parents with poorly controlled diabetes. Diabetes 1978; 27: 889-94.

20. Rayfeild EJ, Heush GT, Gilbert HS, Kovacs I, Smith H. Does diabetic control affect susceptibility to infection. Clin Res 1978; 26: 425A.

21. Drachman RH, Root RK, Wood WB. Studies on the effect of experimental diabetes mellitus on antibacterial defence I: demonstration of a defect in phagocytosis. J Exp Med 1966; 124: 227-32.

22. Tan JS, Watanakuranakuran G, Phair JP. Host resistance in diabetes: Neutrophil dysfunction J. Clin Invest 1972; 51: 169A.

23. Johnson JE. Infection and diabetes. In: Ellenberg M, Rifkin H, Eds. Diabetes Mellitus: Theory and practice. New York: Mc Graw Hill, 1970.

24. MacGuish AC, Urbaniak SJ, Campbell CJ et al. Phytohaemagglutinin transformation and circulating lymphocyte subpopulation in insulin-dependent diabetic patients. Diabetes 1974;23: 708-12.

25. Hunt TK. The physiology of wound healing. Ann Emerg Med 1988; 17: 1265-73.

26. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic: a comparision of the effects of inspired oxygen concentration and antibiotic administration

on in vivo bacterial clearance. Arch Surg 1986; 121: 191-5.

27. Hill GB, Osterhout S. Experimental effects of hyperbaric oxygen on selected clostridial species I. In-vitro studies. J Infect Dis 1972;125 : 17-25.

28. Boehm DEEPAK THORAT, Vincent K, Brown OR. Oxygen and toxicity inhibition of amino acid biosynthesis. Nature 1976;262: 418-20.

29. Brown OR. Reverse inhibition of respiration of Escherichia coil by hypoxia. Microbiol 1972; 5 : 7-16.

30. Park MK, Muhvich KH, Myers RAM, Marzella L. Hyperoxia prolongs the aminoglycoside-induced post anti-biotic effect in Pseudomonas aeruginosa. Antimicrob Agents Chemother 1991; 35: 361-5.

31. Caputo GM, Cavanagh PR, Ulbrecht JS, gibbons JS, Karcher AF: The NEJM Vol 331, 1994, No. 13 : 854-60.

32. Ganda OP/ Pathogenesis of microvascular disease in the human diabetic. Diabetes, Vol 29, Nov. 1980; 930-41.

33. Pendsey S. Diabetic foot-more than a pedestrian problem! NovoNordisk Diabetes Update 95, Proceedings, Health Care Communications, Mumbai –69. 1995: pp 80-5.