

Effectiveness Of Anti-Inflammatory Property of Hyperbaric Oxygen Therapy in Acute Wounds: A Microbiological, Pathological and Clinical Correlation

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Abstract--Orthopedic trauma especially open fractures are associated with increased use of hospital resources, social challenges, and financial loss. Hyperbaric oxygen (HBO) is an adjunct to reduce wound related complications and thus improve outcomes. In the present study, the effectiveness of anti-inflammatory property of HBO in acute wounds were analysed by infrared thermography, microbiological analysis and inflammatory markers. The results show that the HBO therapy is effective and can act as an adjunct for wound healing. A prospective observational study including 80 patients who were divided into two groups. C-Reactive Protein (CRP) and microbiological analysis and peri wound skin temperature monitoring were done on day 0, 4 and 6 among these groups. All patients underwent appropriate surgical procedures and complications were managed as per standard treatment protocols. Our study included 80 patients with acute wounds. Mean age in HBOT group was 39 and in non HBOT group was 41. Mean value of CRP and mean value temperature in HBOT group showed a descending trend in day 4 and day 6 as compared to non HBOT group but an ascending trend was noted in nonHBOT group. Microbiological analysis did not show any microbial growth in HBOT group on day 6 while 8 patient's developed microbial growth in nonHBOT group. HBOT can act as an adjunct to enhance wound healing and to improve the final outcome of acute traumatic wound which is evident from this study. We suggest the use of HBOT in all indicated acute injuries to reduce the socioeconomic burden and to obtain early functional status of patient. Early wound debridement, repair and reconstruction should be done hand in hand with HBOT to achieve the goal.

Key words: HBOT, infrared thermography, CRP, adjunct, acute wound

1. Introduction

Orthopaedic trauma is responsible for a high level of social and personal cost as well as increased use of hospital resources. Open fractures are of particular importance as they present more complex management challenges and are associated with higher risk of complications¹. Hyperbaric oxygen (HBO) can be used as an adjunct to reduce the complications of orthopaedic injury and thus improve outcomes. The committee on hyperbaric medicine defines hyperbaric oxygen therapy as “a mode of medical treatment in which the patient is entirely enclosed in a pressure chamber and breathes 100% oxygen at a pressure greater than 1 atmosphere absolute (ATA).”²

The typical HBO treatment takes place at a pressure of 2.0 to 2.4 ATA. The duration of the treatment depends on the indication but is generally 90 to 120 minutes long and 1 to 2 sittings per day, 5 days a week. The longevity of the treatment depends on the indication. Major indications include infected wounds like clostridial myonecrosis, necrotising soft tissue infections, Fournier's gangrene, traumatic wounds, crush injury, compartment syndrome, compromised skin grafts and flaps and thermal burns³.

Wound healing is a highly integrated process having phases such as hemostasis, inflammation, proliferation, and tissue remodeling or resolution. Interference with one or more phases in wound healing process, results in impaired tissue repair⁴. Various inflammatory markers such as IL6 and CRP can be used to monitor wound healing⁵.

Microbiological analysis of wound is to determine the clinically significant isolates and to perform antimicrobial susceptibility testing⁶. This will provide guidance to the appropriate treatment and to control the antibiotic usage, hence the spread of antibiotic-resistant bacteria⁷.

Increase in vascularity is a hallmark feature of many pathological changes such as inflammation due to increased metabolic activity. This will lead to increase in temperature, which can be detected by using an infrared thermographic camera called as dynamic infrared thermography (DIRT).⁸

In our study, we analysed the effect of Hyperbaric oxygen therapy (HBOT) in improving the outcome of acute wounds in various orthopaedic trauma using inflammatory parameter like CRP and culture & sensitivity as a microbiological parameter and as an adjunct, infrared thermography. The purpose of the present study is to find the usefulness of HBOT in acute trauma using various parameters so that it can be used as an adjunct to reduce the morbidity among patients with acute traumatic injuries. The present study was conducted to evaluate the effectiveness of anti-inflammatory property of hyperbaric oxygen therapy in acute wounds by microbiological analysis and inflammatory marker. Also, to determine the effect of anti-inflammatory property of hyperbaric oxygen therapy in improving the clinical outcome of patients with acute wounds using thermoradiography.

2. Methodology

This was a comparative observational study between HBOT and non HBOT groups. Patients coming to the emergency department of a tertiary centre with acute trauma

meeting the inclusion criteria were studied and clinically evaluated. Based on the mean and standard deviation of pathological markers observed in a pilot study with 95% confidence level and 80% power, minimum sample size comes to 40 in each group. Wound cleaning and debridement were done under adequate analgesia. Infrared thermographic imaging, CRP and microbiological analysis were done as a baseline on day 0. According to surgeons' decision patient is subjected to six sessions of HBOT after adequate surgical fixation of the injury. Culture and sensitivity swab, Infrared thermographic imaging and CRP were repeated on 4th and 6th days in both groups.

Criteria of inclusion (COI):

Minimum age 18 years.

Permission to enroll patient granted by the trauma team managing the patient.

Persons who suffered an acute open fracture were included on the basis of Gustilo Anderson classification (type 1, 2, 3a, 3b and 3c) or with severe soft tissue injury.

Criteria of exclusion (COE)

Hemodynamic instability, resuscitation/intensive care requirements as judged by the trauma surgeon

Patients suffering advanced medical conditions where the severity of the condition was such that the patient was not expected to survive for the follow-up period.

Patients suffering from psychiatric disorders.

End-stage peripheral vascular disease, problems which would be expected to progress to amputation.

Instrument specification

We used monoplace hyperbaric chamber for the therapy. A hand-held digital infrared thermographic camera was used to monitor the periwound skin temperature. This is a non-contact, non-invasive, quick, economic and does not cause any pain to the patient. It is a relatively straightforward non-radiographic imaging approach that detects the temperature variation on the human skin surface. It is a dynamic imaging technique which yields 2D images where each pixel represents the temperature value, from which different region of interest can be studied. We used the Therma CAM™ Reporter 2000 Professional software to complete a report. A reference temperature scale in the form of a color gradient is given adjacent to each infrared image to compare temperature. The blue and blue-green areas represent lowest temperature, yellow-red areas represent higher temperature and red-white areas represent highest temperature. Increase in temperature is directly proportional to degree of inflammation.

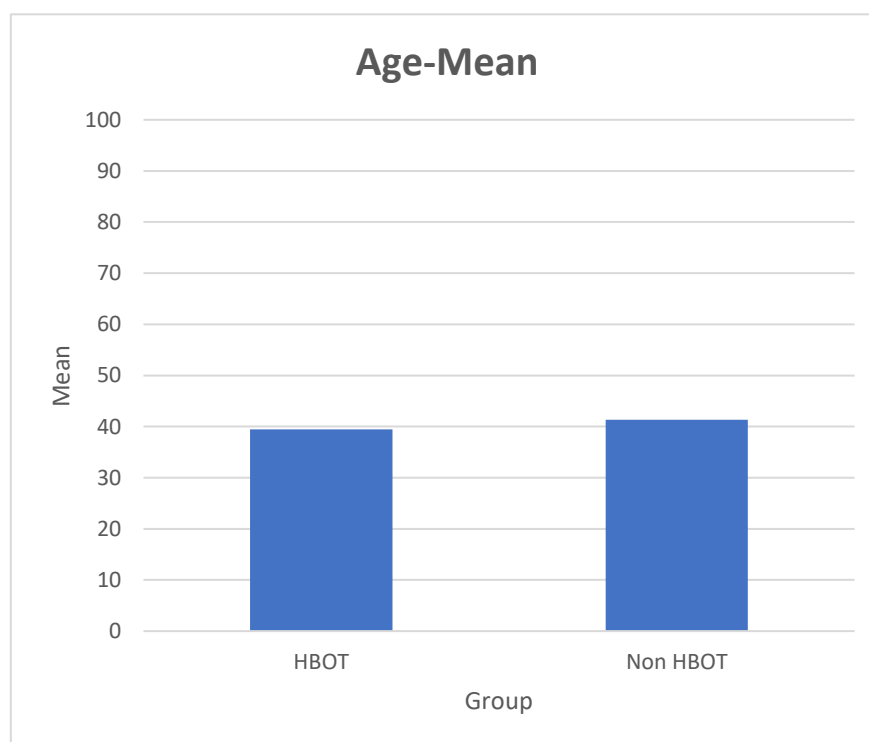
Results on continuous measurements are presented on Mean $\pm$ SD (Min-Max) and results on categorical measurements are presented in Number (%). Student t test (two tailed, unpaired) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis), chi square test was used for categorical variables. Student t test (two tailed, paired) was used to find the significance of study parameters on continuous scale within each group. Significance is assessed at 5% level of significance ($p < 0.05$). The following assumptions on data are made. Data entered into Microsoft excel and analysed using statistical software IBM SPSS version 22. Microsoft Excel 2010 has been used to generate the master chart, tables and graphs.

3. Results

On comparing the age, in HBOT group mean age was 39.43 ± 17.61 and non HBOT group mean age was 41.30 ± 16.67 .

Table1.Comparison of mean age between 2 groups

Groups	N	Age		P Value
		Mean	SD	
HBOT	40	39.43	17.61	0.729
non HBOT	40	41.30	16.67	



Graph 1: Mean age in 2 groups

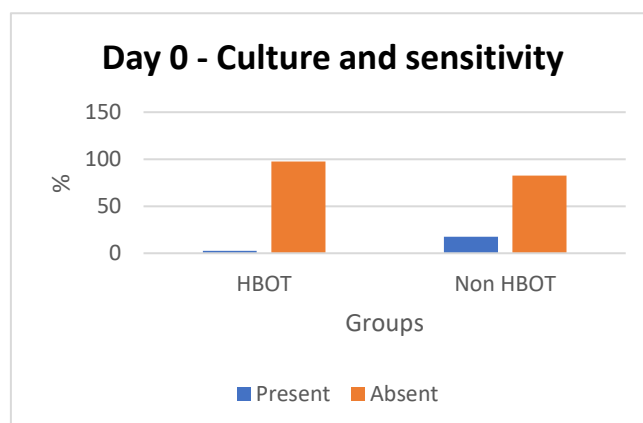
3.1. Comparison of microbiological parameter: culture and sensitivity among HBOT and Non HBOT group

Culture and sensitivity test in HBOT group on day 0, only one sample (2.5%) showed bacterial growth. On 4th day, 2 samples (5%) showed bacterial growth. On 6th day no

sample had presence of organism. In non HBOT group 7 samples (17.5%) had presence of organism. On 4th day, 17 samples had grown bacteria and on day 6, 8 samples (10%) had presence of organism and 32 samples (90%) didn't grow any microbes.

Table 2. Comparison of culture & sensitivity among 2 groups (HBOT and Non HBOT) on the day of injury

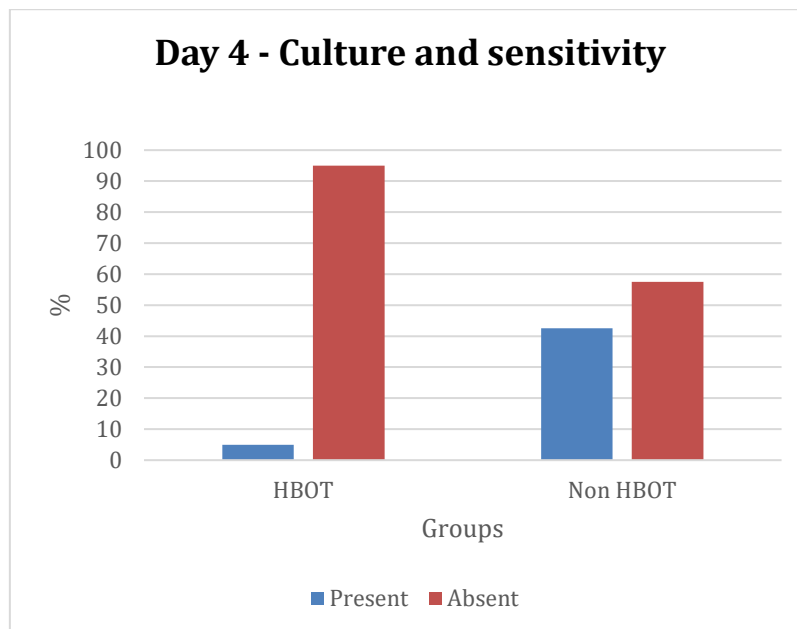
CS Day 0	Groups				χ^2 Value	p Value
	HBOT		non HBOT			
	n=40	%	n=40	%		
Present	1	2.5	7	17.5	5.000	0.025
Absent	39	97.5	33	82.5		



Graph 2: comparison of microbiological parameters among 2 groups on day 0

Table 3 Comparison of culture & sensitivity among 2 groups (HBOT and Non HBOT) on day 4

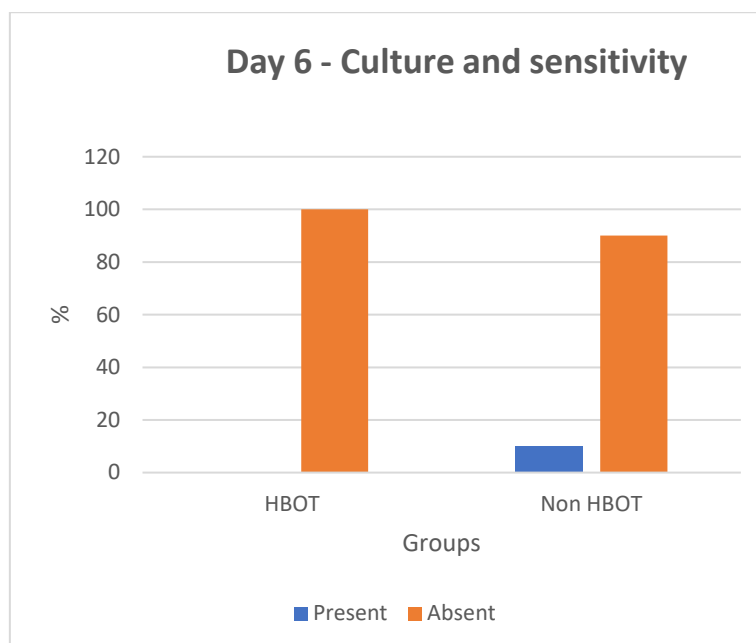
CS Day 4	Groups				χ^2 Value	p Value
	HBOT		non HBOT			
	n=40	%	n=40	%		
Present	2	5.0	17	42.5	15.531	<0.001
Absent	38	95.0	23	57.5		



Graph 3: comparison of microbiological parameters between 2 groups on day 4

Table 4. Comparison of culture & sensitivity *between two groups* (HBOT and Non HBOT) on day 6

CS Day 6	Groups				χ^2 Value	p Value
	HBOT		non HBOT			
	n=40	%	n=40	%		
Present	0	0.0	8	10.0	8.889	0.003
Absent	40	100.0	32	90.0		



Graph 4: comparison of microbiological parameters among 2 groups on day 6

3.2. Comparison of ESR among HBOT and Non HBOT group

Table 5. Comparison of ESR among 2 groups on day 0,4 and 6

ESR	n	HBOT		non HBOT		p Value
		Mean	SD	Mean	SD	
Day 0	80	15.48	7.76	16.48	6.11	0.130
Day 4	80	48.03	12.21	53.93	18.71	0.003
Day 6	80	41.28	10.85	58.35	18.43	0.001

On day 0, mean value of ESR in HBOT group is 15.48 ± 7.76 and mean value of ESR in non HBOT group is 16.48 ± 6.11 . p value was calculated to be 0.130 which is not statistically significant (p value>0.05). On day 4, mean value of ESR in HBOT group is 48.03 ± 12.21 and mean value of ESR in non HBOT group is 53.93 ± 18.71 . p value calculated as 0.003 which is statistically significant (p value<0.05). On day 6, mean value of ESR in HBOT group is 41.28 ± 10.85 and mean value of ESR in non

HBOT group is 58.35 ± 18.43 . p value calculated as 0.001 which is statistically significant. (p value<0.05)

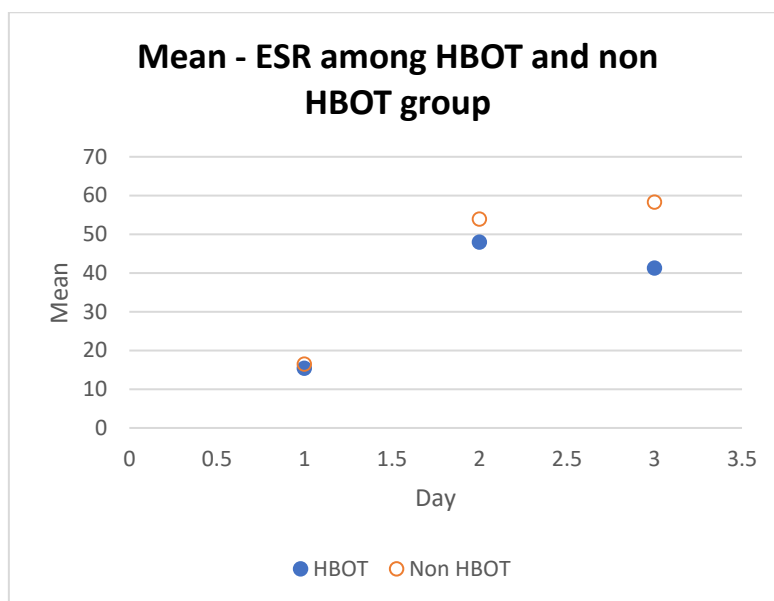


Figure1: line diagram comparing ESR among 2 groups on day 0,4 and 6

3.3 Comparison of CRP among HBOT and non HBOT group

On day 0, mean value of CRP in HBOT group was 0.963 ± 0.36 and mean value of CRP in non HBOT group 1.06 ± 0.52 . On 4th day, mean value of CRP in HBOT group was 3.88 ± 1.18 and mean value of CRP in non HBOT group was 5.79 ± 3.80 . p value calculated from these results was < 0.001 which is statistically significant. On 6th day, mean value of CRP in HBOT group was 2.74 ± 1.93 and mean value of CRP in Non HBOT group is 6.23 ± 3.89 . On comparison p value was calculated as <0.001 which is statistically significant.

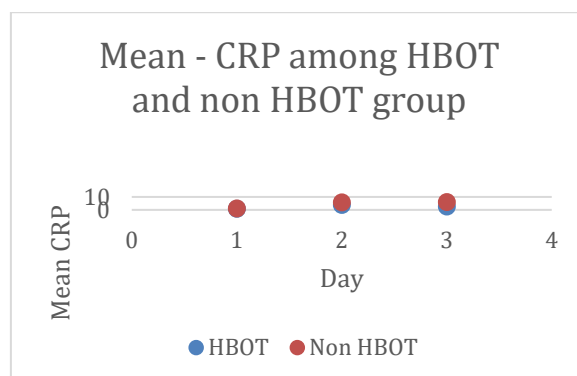


Figure2: Line diagram showing comparison between CRP among 2 groups (HBOT and NonHBOT) on day 0, 4 and 6

3.4 Comparison of the clinical parameter: Temperature measured by digital infrared thermography among HBOT and non HBOT group

Temperature was measured using digital infrared thermography in HBOT and non HBOT groups on day 0, 4 and 6. On day 0, mean value of temperature in HBOT group was 32.66 ± 2.59 and mean value of temperature in Non HBOT group was 32.45 ± 2.45 . p value was calculated to be 0.967 which is statistically not significant. On day 4, mean value of temperature in HBOT group was 34.28 ± 1.52 and mean value of temperature in non HBOT group was 35.07 ± 2.07 . p value was calculated as 0.039 which is statistically significant. On day 6, mean value of temperature in HBOT group was 32.58 ± 1.26 and in non HBOT group was 36.01 ± 2.27 p value was calculated as 0.023 which is statistically significant.

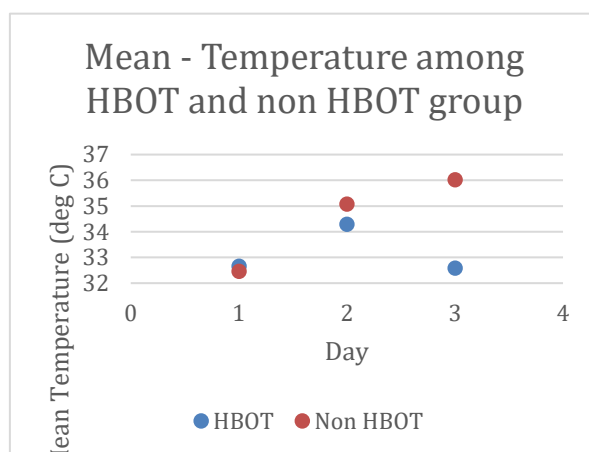


Figure 3: Line diagram showing mean temperature values of HBOT and Non HBOT groups on day 0, 4 and 6

3.5 Correlation between CRP and Temperature between 2 groups

Correlation between CRP and Temperature is statistically significant on day 4 and day 6 among 80 patients.

Table 6: Correlation between CRP and Temperature among HBOT and Non HBOT groups on day 0,4 and 6

Day	N	Correlation Coefficient	p Value
1	80	0.268	0.016
4	80	0.679	<0.001
6	80	0.751	<0.001

4. Discussion

Acute traumatic wound related problems are still a major challenge for the treating surgeon. Hyperbaric oxygen therapy is a capable tool solving these issues to enhance the wound healing. The current study included 80 patients with acute wounds in which 40 received HBOT according to the decision of the treating surgeon and the other 40 did not receive HBOT and will serve as a control arm.

Latham et al. suggested that HBOT should be started as soon as possible, ideally within 4 to 6 hours after injury or surgery at 2 to 2.5 ATA for 60 to 90 minutes. The patient should receive HBOT 3 times daily for 2 to 3 days, followed by twice daily for 2 to 3 days, and finally daily for 2 to 3 days (9). We started HBOT sessions as soon as possible for all patients who met the inclusion criteria and HBOT was done for a minimum of one or two sessions per day for six days and extended according to the indication.

The clinical significance of the microbial load in delaying wound healing was described in 1964 by Bendy et al. The role of microbiology is to determine the antimicrobial susceptibility testing to provide guidance on the most appropriate treatment. It controls the antibiotic usage and hence the spread of antibiotic-resistant bacteria⁷. The most dominant microbes in open fracture wounds include Staphylococcus, Corynebacterium, Streptococcus, Acinetobacter and Pseudomonas¹⁰. In our study, the most common microbe we found was Staphylococcus.

Hyperbaric oxygen therapy neutralizes alpha exotoxins produced by bacteria such as Clostridium and promotes neutrophil-mediated bacterial killing ability in hypoxic tissue¹¹. Current study analysed growth of microorganism by culture and sensitivity among HBOT and non HBOT group and showed that there was no growth of organism

among HBOT group on day 6. In non HBOT group, 8 samples showed microbial growth.

Peri wound skin temperature and wound healing have an association corresponding to the inflammatory stage of healing. An increased skin temperature was detected for the first 3 days and it gradually decreased between days 4 and 6. Persistence of increased skin temperature after the third postoperative day was considered to be a sign predictive of infection and disturbed healing.

This was a prospective observational study with total duration was 18 months. Current study shows promising results in the effectiveness of anti-inflammatory property of hyperbaric oxygen therapy in acute wounds using microbiological parameter, inflammatory marker and infrared thermography.

5. Limitations Of Current Study

Patients were selected on random basis according to the inclusion criteria. Age, sex, adjunct therapies and antibiotics were not taken into consideration. Environmental factors can affect the thermography reading, which was analysed by comparing with contralateral limb. One incidental finding which we observed that patient who had early soft tissue healing with HBOT had less soft tissue morbidity, but this requires further evaluation. IL6 is a specific marker for inflammation, which is not included due to unavailability and high cost. Comorbidities of the patients were not taken into consideration except end-stage peripheral vascular disease.

6. Figures

HBOT CASE

A 47-year-old male came with history of trauma to left leg. Diagnosed as a compound fracture of calcaneum with severe soft tissue injury. Debridement and closure of wound done. Fracture was stabilized with an external fixator and multiple K wires. On the postoperative day 1, HBOT was started as single session per day for 6 days. Infrared thermography images and CRP were done on day 0, 4th and 6th day. Thermography images showed a temperature of 36.7°C on 4th day and 34.7°C on 6th day. Wound was looking healthy with no signs of infection or necrosis. Patient was discharged on 7th day. No secondary procedures were done.



Figure 4.1: Showing compound fracture of calcaneum with severe soft tissue injury on the day of injury



Figure 4.2: Infrared image of ankle and foot showing periwound temperature on the day of injury



Figure 4.3: Clinical photo of limb on post-operative day 4



Figure 4.4: infrared thermography image showing more red areas on post-operative day 4



Figure 4.5: Clinical photo of the limb on postoperative day 6



Figure 4.6: Infrared thermography image showing reduction of red areas on postoperative day 6

NON HBOT CASE

A 35-year-old female came with history of slip and fall and sustained an injury to dorsal aspect of her left ankle. On examination, there was a deep lacerated wound with contamination and tendo Achilles was completely torn. Debridement and closure of wound was done on day 0. On postoperative day 4, wound was found to be clean but edema was not reduced. Infrared thermography images were taken on preoperative day,

postoperative days 4 and 6 and temperature was also noted. ESR and CRP was done on these days. Thermography images showed a temperature of 35.5°C on day 4 and 35.8°C on day 6. ESR and CRP values showed no significant reduction. Wound was looking healthy but edema was persisting. Patient was complaining of continuous aching type of pain. Antibiotic was escalated. Patient was discharged on day 10. No secondary procedure was needed.



Figure 5.1: day 0 clinical photo showing lacerated wound over the dorsal aspect of left ankle with completely torn tendo Achilles

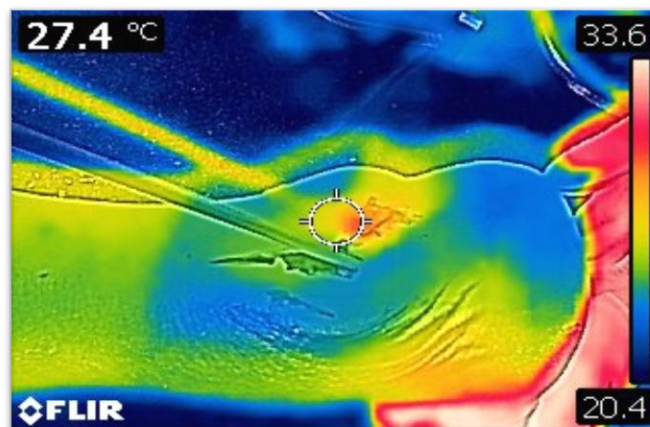


Figure 5.2: day 0 infrared photo showing yellow colour over the injured area



Figure 5.3: day 4 clinical photo showing sutured wound with repaired tendon immobilized in a slab

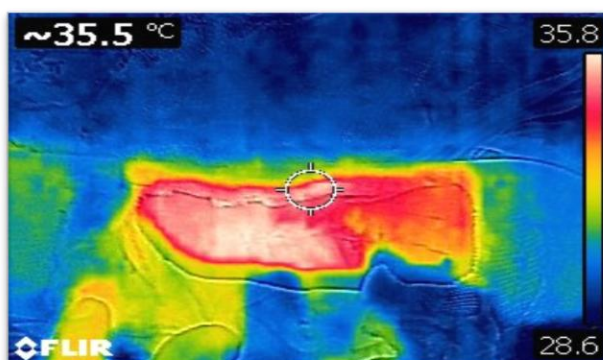


Figure 5.4: day 4 infrared photo showing more red and white areas



Figure 5.5: day 6 clinical photo showing mild increase in swelling around the wound

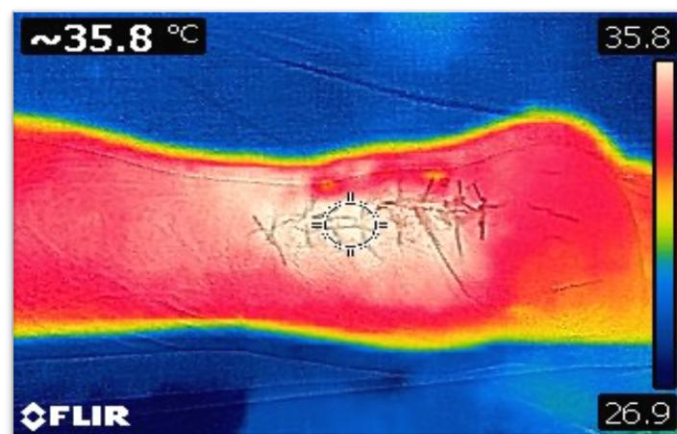


Figure 5.6: day 6 infrared photo showing increased white and red areas

7. Conclusion

Hyperbaric oxygen therapy is an adjunct to reduce the wound related complications of musculoskeletal injury and to improve the outcome. CRP is a principal downstream mediator of the acute-phase response following an inflammatory event and is primarily synthesized by IL-6-dependent hepatic biosynthesis. The CRP will return to normal, within a day or so, if the focus of inflammation is eliminated. There is a significant role for microbial load in delaying wound healing. The role of microbiology is to determine the antimicrobial susceptibility testing to provide guidance on the most appropriate treatment. InfraRed thermography (IRT) has become an increasingly preferred adjunct to more expensive and/or invasive investigations. Inflammation will result in increased metabolic activity, increase in vascularity, thus increase in temperature, which can be detected by an infrared thermographic camera. BOT is an effective adjunctive therapy for all acute wounds to improve the outcome. Further studies will continue to expand on the cellular effects of the hyperoxia. Now HBOT has become more accessible to patients and institutions, which will improve the cost-effectiveness and convenience and create better opportunities for patients with acute wounds.

8. References

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