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International Conference on Physical Instrumentation and Advanced Materials

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Preface

The International Conference on Physical Instrumentation and Advanced Materials (ICPIAM) took place in the Hotel Santika Premiere Surabaya, on October 27, 2016 and was organized by the Department of Physics, Faculty of Science and Technology, Airlangga University, Surabaya Indonesia.

This conference will conduct biannual regularly. The main objective of this conference is to bring together students, researcher and practitioners of physical instrumentation and advanced material to share and disseminate the research result.

We are fortunate to have world-known keynote speaker: Prof. Tamio Oguchi and Prof. Yoshitada Morikawa (Osaka University), Prof. Sulaiman Wadi Harun (Malaya Uniersity), Prof. Moh. Yasin and Febdian Rusydi, Ph.D. (Airlangga University), who are willing to share their perspectives on the current status of researches.

We would like to thank the member of local organizing committee who help with all the preparations required to make the conference success. Of course, special thanks also go to the reviewers, the authors and all of the participants for their contributions in making ICPIAM 2016 successful.

In addition, we would also thanks to Science and Technology Faculty and Institute of Research and Innovation Airlangga University in which, without their support we would not able to organize this conference. I believe that we will have high quality discussion in order to increase our excellence in scientific area of physical instrumentation and advanced material. I also hope that this will become a stepping stone of potential collaboration between academicians and professionals in Indonesia in the future.

Finally, the success of this conference lies not only in the quality of papers presented but also to a large extent upon the dedicated team efforts of the many volunteers, in particular members of the Organizing Committee and International Advisory Committee. Their dedicated contribution and encouragement have been exemplary. We would also like to thank the staff at Integrated Meetings Specialist, who have given their best to ensure the smooth running of the conference.

Last but not least, I would like to acknowledge with sincere thanks to our sponsors and supporters. To all our delegates, I hope the 1st ICPIAM 2016 will be memorable not only from the scientific perspective but in the joy of meeting old friends and making new ones.

Dr. Khusnul Ain
General Chair of ICPIAM 2016

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Peer review statement

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Peer review statement

All papers published in this volume of *Journal of Physics: Conference Series* have been peer reviewed through processes administered by the proceedings Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

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Effects of hyperbaric oxygen therapy in enhancing expressions of e-NOS, TNF-α and VEGF in wound healing

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Abstract. Wound healing is a physiological process that occurs progressively through overlapping phases. Tissue oxygenation is an important part of the complex regulation for wound healing. Hyperbaric Oxygen (HBO) therapy is a method of increasing oxygen delivery to tissues. The therapy improves tissue oxygenation and stimulates the formation of H2O2 as a secondary messenger for Tumour Necrosis Factor alpha (TNF α), e-NOS, VEGF and Nuclear Factor Kappa Beta phosphorylation (NF-Kb) which play an important role in the rapid transcription of a wide variety of genes in response to extracellular stimuli. This study aims to determine the effects of Hyperbaric Oxygen therapy in enhancing the expressions of e-NOS, TNF-α, VEGF and wound healing. This study is an animal study with a 'randomized control group of pre-test and post test design' on 28 Wistar rats. Randomly, the rats were divided into 4 groups with 7 rats in each group. The HBO treatment group 1 received 5 sessions of HBO 2.4 ATA in 3x30 minutes; the HBO treatment group 2 received 10 sessions of HBO 2.4 ATA in 3x30 minutes; and each of the control groups were without HBO. Each of the 28 male rats were given a full thickness excisional wound of 1x1cm. Examinations of e-NOS, TNF-α, VEGF expressions and wound healing were performed on day-0 (pre-HBO) and day-5 HBO or on day-0 (pre-HBO) and day-10 HBO.The resultsshowthat the Hyperbaric Oxygen therapy can improve e-NOS (p=0.02), TNF-α (p= 0.02), VEGF expression (p=0.02) and wound healing (p=0.002) significantly in the provision of HBO 2.4 ATA for 3x30 minutes in 5 sessions over 5 consecutive days. While the 10 sessions of HBO 2.4 ATA for 3x30 minutes over 10 consecutive days only increase e-NOS (p=0.02), TNF-α (p=0.04), VEGF expression significantly (p=0.03) but do not improve wound healing significantly (p=0.3) compared with no HBO. The study concludes that HBO can improve the expressions of e-NOS, TNF-α, VEGF and wound healing in the provision of HBO 2.4 ATA for 3x30 minutes in 5 sessions, while the 10 sessions of HBO 2.4 ATA for 3x30 minutes only increase e-NOS, TNF-α, VEGF expression but do not improve wound healing.

1. Introduction

Wound healing is a normal process through a highly complex mechanism. The process occurs progressively through overlapping phases; haemostasis, inflammatory, proliferation, contraction and tissue remodelling [1]. The healing process can be ended by fibrosis or scarring even in optimal conditions, resulting in multiple disabilities [2] Acute injuries can become chronic wounds. The problem of chronic wounds is now a challenge for modern society resulting economically and socially significant medical impact. A Chronic wound is one of the most frequent causes of morbidity in developing countries [3].

An estimated 1.5% incident of the population suffered injuries at any points in time. Laceration or wound often encountered daily (estimated at 20 million cases each year) as a result of cut. A post operation wound is the biggest cause of skin trauma. There are more than 110 million surgical incisions every year, some which can't recover completely due to various causes. The wound developed into chronic wounds .The large number of the population and the burden of costs require serious attention on early detection, prevention, diagnosis and treatment of chronic wounds [2].

The Hyperbaric Oxygen therapy, as an adjuvant therapy in wound healing, results an increase in RNS mainly in NO through iNOS and e-NOS, as well as an increase in ROS, especially H2O2. Excessive doses of RNS and ROS are a threat to the survival of the cells, while the therapeutic doses (2.4 ATA) of HBO provide positive benefits for wound healing [4]. In clinical use, the dose of HBO is restricted from 2-2.5 ATA to a maximum of 3 ATA for 60-120 minutes. The HBO therapy varies between 5-30 times [5]. The provision of 100% oxygen at a pressure of 2 ATA improves tissue oxygenation of 30-40 mmHg in normal circumstances up to 250-300 mmHg, while the pressure of 3 ATA allows the improved tissue oxygenation up to 10-15 times [6].The Hyperbaric Oxygentherapy increases tissue oxygenation and stimulates the formation of H2O2 as a secondary messenger for the phosphorylation of Nuclear Factor Kappa Beta (NF-kB). The Nuclear Factor Kappa Beta/Rel transcription proteins play an important and quick role on a wide variety of genes in response to extracellular stimuli. Many aspects of the inflammatory response are also regulated by NF-kB, including the production factors of complement cascade and the induction of proinflammatory cytokines such as IL1 and TNF α [7]

The Tumour Necrosis Factor (TNF) group consists of 19 cytokines which have an important role in regulating the development and functions of the immune system. Cytokines from the group of Tumour Necrosis Factor (TNF) regulates the inflammatory and immune processes. The lack of TNF can trigger cell deaths. Although TNF-α cytotoxicity affects some tumour cells, TNF α will only trigger apoptosis if the NFkB signalling is inhibited. TNF α is an activator ofNFkB, proinflammatory transcription factors that help cells survival through activation of pro-survival genes and suppression of pro-apoptotic genes.

Neovascularization holds a central function in wound healing. In vitro studies indicate that VEGF, the most potent angiogenic protein, was induced by NO. Barriers will disrupt NO synthesis of VEGF expression and angiogenesis, thus slowing down the process of wound healing.

The Hyperbaric Oxygen therapy suppresses the inflammatory response to prevent chronic wounds [8] Supplementary oxygen through the blood stream accelerate sangiogenesis in ischemic wounds. Therefore, accelerates the healing time [6]. Numerous studies have proven the benefits of ATA 2.4 HBO five sessions and ten sessions [9]

Our previous studies result that HBO increases the expression of iNOS ($p = 0.001$), while the wound healing ($p = 0.002$) significant at 2.4 ATA HBO administration for 3x30 minutes in 5 sessions. Furthermore, the provision of 2.4 ATA HBO for 3x340 minutes in 10 sessions only enhance the expression of iNOS significantly ($p = 0.002$) but does not improve wound healing significantly ($p = 0.3$) compared with no HBO administration [10].

This study aims to determine the influence of the Hyperbaric Oxygen therapy (HBO) to increase the expression of TNF-α and its relationship with the increased expressions of e-NOS, TNF-α and VEGF in wound healing. This study tests the influence of hyperbaric oxygen therapy (HBO) to the

increase of eNOS, TNFα and VEGF expression by the immune histochemical examination and the macroscopic wound healing process by measuring the area of wound closure on Rattusnorvegicus Wistargallus. The results are expected to be a reference for drafting standard operational procedures (SOPs) of the use of hyperbaric oxygen therapy (HBO) for wound healing in health care centres.

2. Methods

This study was randomized by a controlled group of the pre test-post test designed in 28 Wistar rats. The samples were divided into 4 groups by random allocation, each consists of 7 rats. Treatment group 1 was given HBO 2.4 ATA for 3x30 minutes in 5 sessions; the treatment group 2was given HBO 2.4 ATA for 3x30 minutes in 10 sessions, and each of the control groups were without HBO. Allof the 28 rats were given a 1x1cm full-thickness excisional wound. An immunohistochemical examination of the expressions of eNOS, TNFα, VEGF and the wound size measurement were conducted to determine the wound healing. The immunohistochemical examination and the wound size measurement to the treatment group 1 and the control group 1 were conducted on day 0 (pre-HBO) and day 5 of the HBO therapy. The immunohistochemical examination and the wound size measurement on the treatment group 2 and the control group 2 were conducted on day 0 and day 10 of the HBO.

3. Results and discussion

The study was conducted after the experimental animals had been acclimatized for 7 days. Skin tissue sampling was done by making a full-thickness of 1x1 cm excisional wound on the dorsal part of the experimental animals. The skin tissue sampling on day 0 was performed on all animals (a total of 28 samples) in the treatment group and the control group. The treatment group was given Hyperbaric Oxygen treatment in 5 sessions and 10 sessions. Comprehensive measurement of a wound closure and tissue sampling were done after the treatment of Hyperbaric Oxygen on day 5 (a total of 14 samples) and on day 10 (a total of 14 samples). The slides was made and immunohistochemical staining was conducted.

Immunohistochemical examination for e-NOS, TNF-α and VEGF expressions was conducted under a microscope. This examination was intended to count the number of immunoreactive cells against e-NOS, TNF-α and VEGF. The quantitative data obtained by counting the number of cells in 10 different fields of view at 40x magnification.

3.1. Day 0 (Pre HBO) condition of variables of expressions of e-NOS, TNF-α, VEGF, and wound healing

The examination of e-NOS, TNF-α, VEGF expressions and wound healing done in groups with and without HBO while the extent of pre HBO wound receives the same treatment, i.e. full thickness of1x1cm (100 mm^2)excisional wound size. On day 0 the expressions of e-NOS, TNF-α and VEGF in inflammatory cells with low intensity were observed.

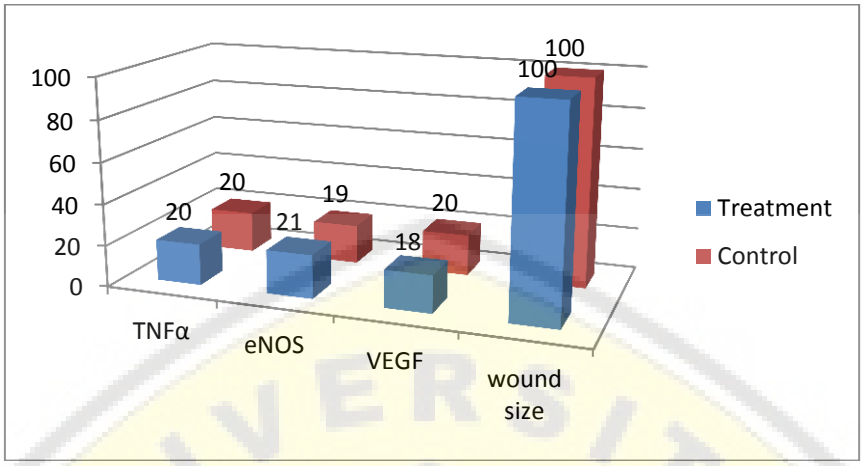

Figure 1. Average HBO on Day 0; Variables of expressions of eNOS, TNFα, VEGF and wound size

3.2. *HBO condition of variables of expressions of e-NOS, TNF-α, VEGF and wound healing on day 5*

The administration of HBO 2.4 ATA for 3x30 minutes in 5 sessions conducted in the treatment group, followed by an examination of e-NOS, TNF-α, VEGF expressions and the wound size to the treatment group and the control group. Figure 2 shows the average results of the variable expressions of e-NOS, TNFα, VEGF and the wound size. On day 5 there are increased of e-NOS, TNF-α, VEGF expressions with high intensity in the treatment group and the control group. The increased expressions of e-NOS, TNF-α, VEGF in the treatment group are higher than those in the control group. Meanwhile, the wound healing in the HBO treatment group is higher than that in the control group (smaller wound size and better healing).

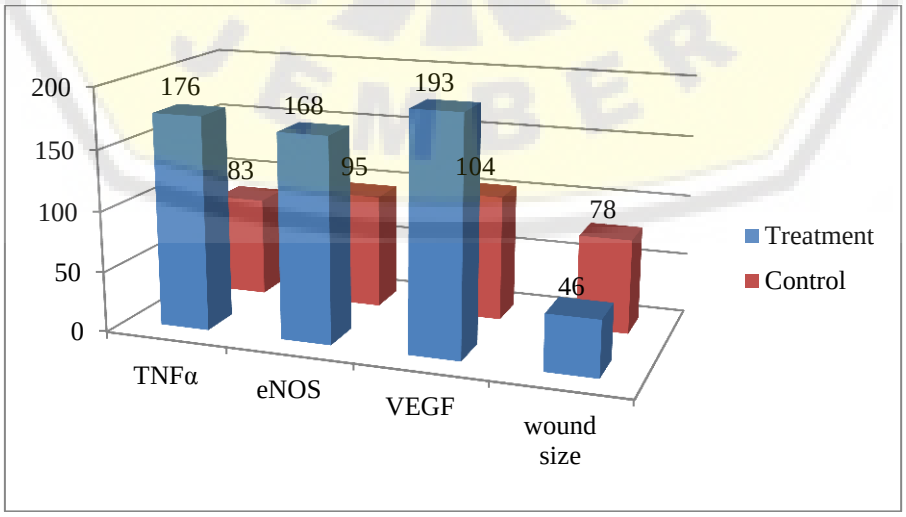

Figure 2 Average HBO on day 5; Variables of Expressions of e-NOS, TNF-α, VEGF, and wound size

3.3. HBO condition on day 10; variables of expressions of e-NOS, TNF-α, VEGF, and wound size

The HBO administration of 2.4 ATA in 3x30 minute each session was conducted 10 times in the treatment group and followed by an examination of the expressions of e-NOS, TNF-α, VEGF and the wound size to the treatment group and the control group of HBO administration on day 10. Figure 3 shows that the intensity of the expressions of e-NOS, TNFα, VEGF were still high but lower than that of on day 5. There is no significant difference of the wound healing variable between the treatment group and the control group.

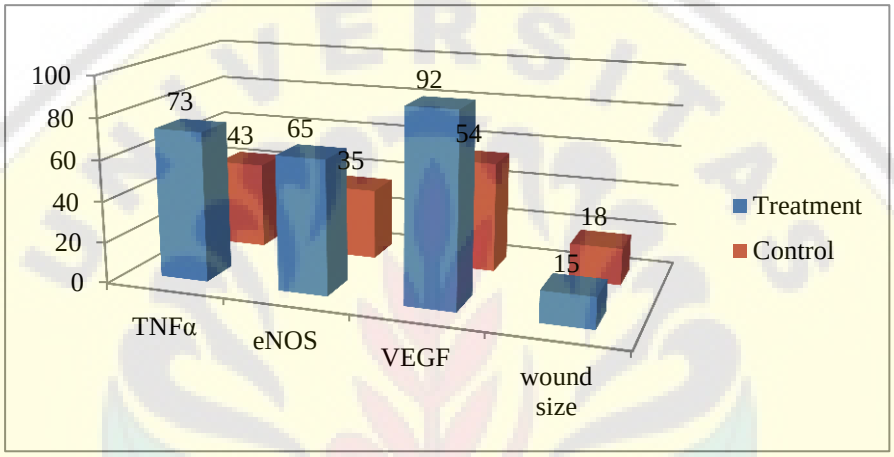

Figure 3. Average HBO on Day 10; e-NOS, TNF-α, VEGF Expressions and Wound size

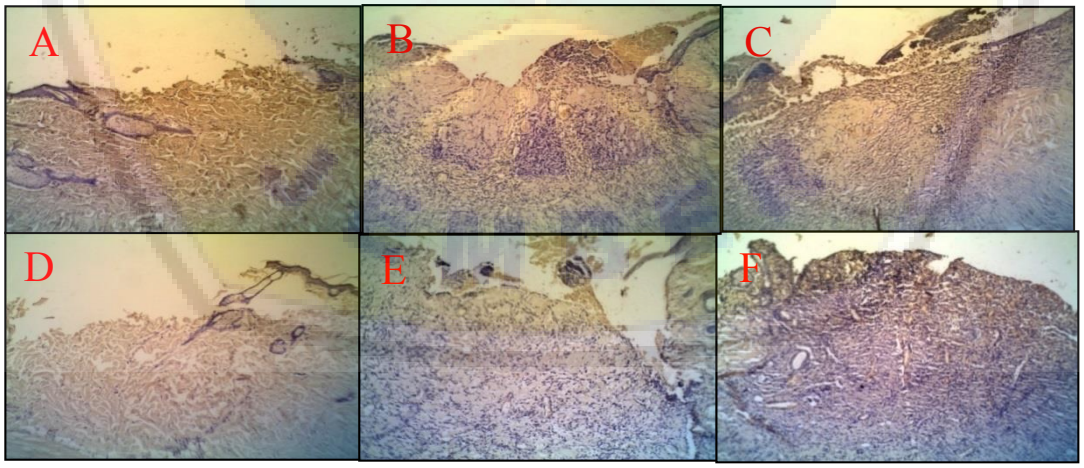

Figure 4. TNFα Expressions using a light microscope Olympus CX31 objective magnification 40x. A: control group day 0, B: control group day-5, C: control group day 10, D: treatment group day 0, E: treatment group day-5, F: treatment group day 10. The intensity of expression of TNF-α is still low in the control group day 0 but the day-5 intensity increases the expression of TNF-α, then the 10^{th} day intensity of the expression of TNFα declines again.

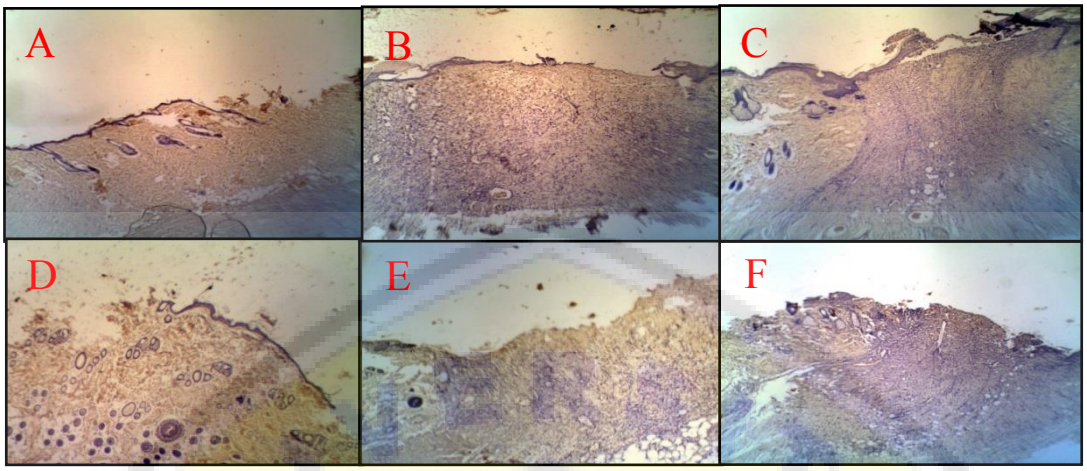

Figure 5. Expressions of e-NOS under a light microscope Olympus CX31 objective magnification 40x. A: control group day 0, B: control group day5, C: control group day 10, D: treatment group day0, E: treatment group day5, F: treatment group day 10. In the control group day 0, the e-NOS expression remains in low intensity; The intensity of e-NOS expression increases on day 5 but declines again on the 10th day.

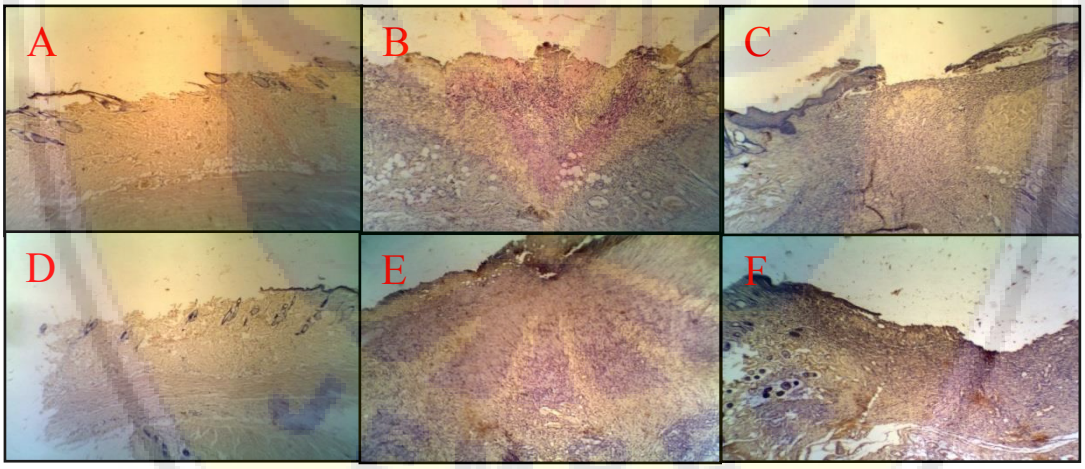

Figure 6. VEGF Expressions under a light microscope Olympus CX31objective magnification 40x. A: control group day 0, B: control group day5, C: control group day 10, D: treatment group day 0, E: treatment group day5, F: treatment group day 10. The intensity of VEGF expression is low in the control group day 0; The VEGF expression intensity increases on the 5th day but decreases again on the 10th day.

The results of examination on the expressions of e-NOS, TNFα and VEGF in the treatment group and the control groups on day0 show that the expressions of e-NOS, TNF-α and VEGF intensity are low (1-1). After the administration of HBO 2.4 ATA in five sessions, on the 5th day there seems to be increased expressions of e-NOS, TNF-α and VEGF (2-2) along with the increased number of inflammatory cells, in which the increased expressions of e-NOS, TNF-α and VEGF in the treatment group is significantly higher than those of the control groups. On the 10th day the intensity of the expressions of e-NOS, TNF-α, VEGF is still high. However,

the number of cells that express them reduces and the number of inflammatory cells remains high (2-1).

TNFα is a cytokine regulator and major effectors, mainly secreted by monocytes and macrophages that have been stimulated [11]. HBO administration increases the production of e-NOS in cultured endothelial cells [12]. Several biological processes and growth factors are stimulated and increased with Hypoxia and HBO. Such processes include angiogenesis, collagen synthesis, the activity of osteoclasts, and the release of VEGF and TNF-α. Oxygen stimulates biological processes under conditions of hypoxia and hyperoxia. It is called Oxygen Paradox. One mechanism for forming collagen fibroblasts is stimulated through peroxide. This occurs in HBO hypoxia wounds and during therapy. Peroxide formed in the HBO therapy resembles the stimulus that occurs during hypoxia. Another mechanism is cytokine stimulation which begins with hypoxia, and increases in cytokine regulation which is supported by the presence of hyperoxia occurring in the HBO therapy. VEGF, TNF-α TGF-β and PDGFβ are released at wound hypoxia as an initial stimulus, but its activity increases in hyperoxia conditions, especially in the presence of lactic acid [13].

The evidence shows that HBO can accelerate wound healing through mechanisms expressions of e-NOS, TNFα and VEGF in the inflammatory phase and the proliferative phase. The administration of HBO can potentially be used for a post-surgical adjuvant therapy for certain cases, thus it is expected to reduce the duration of treatment (Length of Stay / LOS) in the hospital. Indeed, it is expected to reduce the cost and recovery time for patients after surgery so that patients can be immediately productive and return to their normal activities. Therefore, we need to do more research on the effects of hyperbaric oxygen therapy against various types of wounds, including wounds that are conventionally difficult to cure, post-trauma wounds and postoperative wounds.

The evidence also shows that the administration of HBO continuously for a long time does not provide significant benefits for wound healing. Therefore, time off (recovery time) needs to be given after administration of certain HBO in 5 sessions before continuing to the next session. Further research is needed to determine whether or not there are effects of the recovery period and the range of speeds of the wound healing/recovery time interval before the next continuing HBO therapy session. Further research with more dose variations (between 1-5 sessions and between 5-10 sessions) is needed to obtain the optimal dose of the Hyperbaric Oxygen therapy for acute wounds without infections. Further research needs to be conducted to determine the repetition time interval after the administration of the Hyperbaric Oxygen therapy in5 sessions (recovery period) as well as the use of HBO as an adjuvant therapy for various types of injuries.

4. Conclusion

This study concludes that applying 2.4 ATA HBO for 3x30 minutes in 5 sessions increases the expressions of e-NOS, TNF-α, VEGF and accelerates wound healing, while giving HBO 2.4 ATA for 3x30 minutes in 10 sessions enhances the expression of e-NOS, TNF-α and VEGF but does not accelerate wound healing.

Furthermore, the evidence shows that giving HBO can accelerate wound healing through mechanisms of e NOS, TNF-α and, VEGF expression. The HBO administration can be potentially used for a post-surgical adjuvant therapy and specific cases as the administration is expected to shorten the duration of treatment in the hospitals, and reduce the cost and recovery time for patients after surgery so that they can return to normal activities.

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