Serum Albumin Levels of Oral Candidiasis Immunosuppressed Rats Treated with Hyperbaric Oxygen

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Abstract

Objective: To investigate serum albumin levels in oral candidiasis immunosuppressed rats treated with hyperbaric oxygen. One of the predisposing factors for oral candidiasis was the use of immunosuppressive drugs continuously. It can also affect the work of the liver because it's one of the organs responsible for drug metabolism. Hyperbaric oxygen therapy was used not only to suppressing fungal infections, but also to improve liver function by evaluating the serum albumin levels.

Methods: This study used a post-test only control group design. Fifteen Wistar rats were divided into 3 groups(n=5/3): G1 (healthy group), G2 (oral candidiasis immunosuppressed rats group without hyperbaric oxygen therapy), and G3 (oral candidiasis immunosuppressed rats group with hyperbaric oxygen therapy). G2 and G3 groups were immunosuppressed by giving dexamethasone 0,5mg/day/rat orally for 14 days, added with tetracycline 1 mg/day/rat. Hyperbaric oxygen therapy was given to the G3 group in 5 days. Blood serum of rats in all groups was taken to calculate albumin levels.

Results: The average value of albumin levels in G2 group showed a decrease compared to the G1 group, while G3 showed the highest level. One way Anova test showed a significant difference among groups ($p<0,05$). To compare the difference between each group we used LSD test and showed a significant difference ($p<0,05$) between G1 compared to G2, G1 compared to G3, and G2 compared to G3.

Conclusion: Liver albumin levels of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen therapy showed higher levels than those without therapy.

Keywords: Albumin, hyperbaric oxygen, immunosuppressed, liver, oral candidiasis

Introduction

Oral candidiasis is a fungal infection that occurs in the oral cavity. The cause of this infection, as many as 95% of cases are *Candida albicans*.[1] One of the predisposing factors for this infection is the use of immunosuppressive drugs, which can enhance the growing colonies of *Candida albicans*.[2,3]

The liver is one of the organs in the body that liable for drug metabolism. Drugs contained in the blood will be absorbed through the hepatic entero circulation, which will then be metabolized in the liver.[4] In addition to being a predisposing factor for oral candidiasis, continuous use of immunosuppressive drugs can affect the function of hepatocytes as a major part of metabolic agents, which can subsequently cause liver injury.[4,5]

Any abnormalities in the liver can be identified using liver function tests, one of them by evaluating serum albumin. It also can provide information about liver function.[6,7] Changes in its levels more or less than normal, can be a sign of liver damage, and also determine the prognosis of liver disease. The levels of this serum albumin can be used as indicators in hepatocellular function. A decrease in albumin levels can indicate liver disease such as cirrhosis hepatis.[8]

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Hyperbaric oxygen therapy (HBOT) has been reported as one of the therapies that had a significant effect on suppressing fungal infections.[9,10] Previous study showed that hyperbaric oxygen therapy 2.4 ATA which was given 3x30 minutes/day for 5 days, could increase the number of lymphocytes in oral candidiasis immunosuppressed model. It could be used as one of the adjuvant therapy in eliminating oral candidiasis infections.[11] Hyperbaric oxygen therapy was also reported as therapy used in several liver diseases, such as acute liver injury, liver fibrosis, non-alcoholic steatohepatitis, cancer, and also has a beneficial effect on liver regeneration due to the antioxidant and anti-inflammatory effect derived from its mechanism.[12]

Based on the description above, the aim of this study was to investigate serum albumin levels of Wistar rats in oral candidiasis immunosuppressed condition which was treated with hyperbaric oxygen.

Methods

This study used a post-test only control group design. The population of this study was fifteen male Wistar strain rats, 6 months old, weighs 200–250 grams, and divided randomly into three groups: G1 (healthy rats group), G2 (oral candidiasis immunosuppressed rats group without hyperbaric oxygen therapy), and G3 (oral candidiasis immunosuppressed rats group treated with hyperbaric oxygen 2,4 ATA 3x30 minutes/day for 5 days). Immunosuppressed condition in rats was made by giving dexamethasone 0,5 mg/day and tetracycline 1 %/day orally. On the 4th day, we reduce the dose as much as 10% for dexamethasone and tetracycline, then rats were induced with *Candida albicans* (ATCC-10231) 6x10^{8} as much as 0,1 cc, applied on the dorsum tongue of rats using a sterile cotton bud, given once every two days for 12 days.[11,13]

Hyperbaric oxygen therapy was given to the G3 group for five days. During therapy with hyperbaric oxygen, rats were still given tetracycline 0,1 mg/day to prevent bacterial infection. The rats were placed in the mono-place chamber, the pressure was increased to 2,4 ATA, then pure oxygen (100%) was flowed for 3x30 minutes, with intervals breathing normal air for 5 minutes. After that, the pressure was lowered to the initial pressure (1 ATA).[11] The blood of rats in all groups was taken from their heart using a syringe, as much as ± 3cc, then centrifuged to obtain the blood serum for counting albumin levels.

Statistical analyses were done with the Lavene statistical test to perform the homogeneity of the data. We used a statistical one-way Anova test to show the different levels of serum albumin among groups, then the Post Hoc LSD test to show the significant difference among each group. To compare the serum albumin levels between groups that were given hyperbaric oxygen therapy and group which did not, we used an independent sample t-test.

This study was conducted in the oral biology laboratory of Dentistry Faculty Universitas Hang Tuah Surabaya, Faculty of Medicine Universitas Hang Tuah Surabaya, and Balai Besar Laboratorium Kesehatan Surabaya, which was held during August-December 2019. This study has been approved by Ethics Commission of Dentistry Faculty Universitas Hang Tuah Surabaya (No: EC/010/KEPK-FKGUHT/VII/2019).

Table 1 The Mean and Standart Deviation (SD) of Albumin Levels in Each Group

Groups	Albumin (g/dl) Mean±SD
G1 (healthy rats)	3.2±0.12
G2 (oral candidiasis immunosuppressed rats without HBOT)	2.96±0.17
G3 (oral candidiasis immunosuppressed rats with HBOT)	3.4±0.12

Results

The mean value of serum albumin levels in the G2 group showed the lowest, compared to G1 and G3 group, while in G3 showed the highest mean value of serum albumin levels. We used the homogeneity of variances test using the Lavene statistic test and showed the homogeneous data ($p>0.05$). One way Anova test showed a significant difference among groups ($p<0.05$). LSD test was used to compare the difference between each group and showed significance value ($p<0.05$) between G1 compared to G2, G1 compared to G3, and G2 compared to G3. This means that there was a significant difference between G1 group compared to G2, between G1 compared to G3, and between G2 compared to G3. To ascertain the differences between groups of

Table 2 Serum Albumin Levels between Oral Candidiasis Immunosupressed (OCI) Rats Group with and without Hyperbaric Oxygen Therapy

Variable	OCI without HBOT	OCI with HBOT	p value
Serum Albumin (g/dl)	2.96±0.17	3.4±0.12	0.001*

p value was obtained from independent sample t-test

oral candidiasis immunosuppressed rats given hyperbaric oxygen therapy and those which did not, we used an independent sample t-test, and showed a significant difference between groups (sig.2 tailed <0,05).

Discussion

Serum albumin levels of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen 2,4 ATA 3x30 minutes/ day with 5 minutes interval breathing in normal air, given for five days continuously (G3 group), showed the highest levels compared to the G1 and G2 groups. This elevation of serum albumin levels was in line with a previous study which showed that the administration of hyperbaric oxygen therapy could significantly increase serum albumin levels in patients with diabetic foot ulcers.[14] Several studies revealed

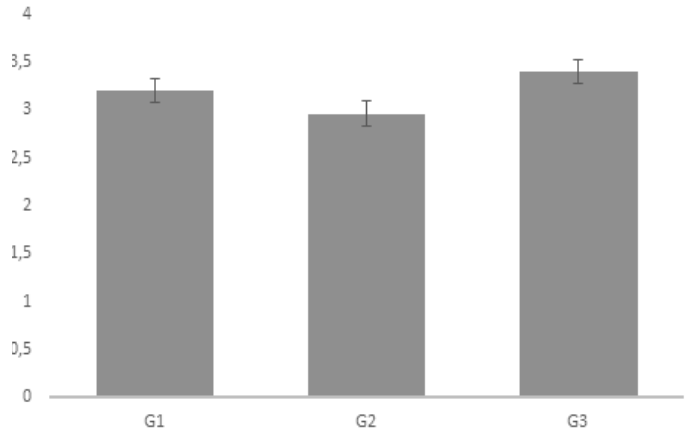

Fig. 1 Graphics of Average Value of Albumin Levels in Each Group. Healthy rats group (G1), Oral Candidiasis Immunosupressed (OCI) Rats Group without Hyperbaric Oxygen Therapy (G2), and Oral Candidiasis Immunosupressed (OCI) Rats Group Treated with Hyperbaric Oxygen 2,4 ATA 3x30 minutes/day for Five Days (G3)

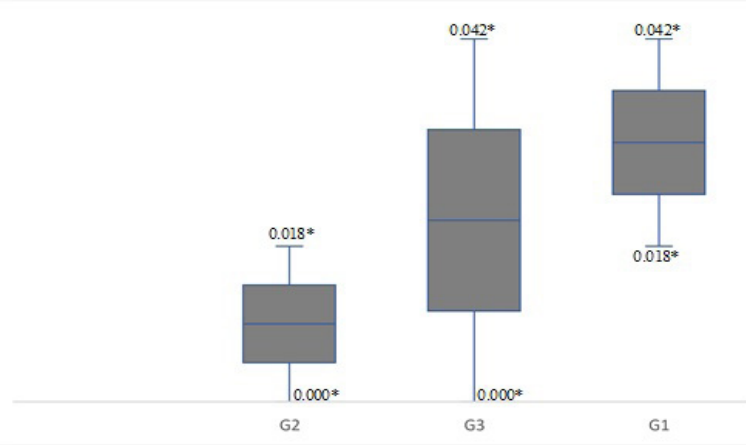

Fig. 2 Diagram of the Significance Value in Each Group Obtained from LSD Test
***significant difference (p<0.05)**

the effect of hyperbaric oxygen therapy on liver disease. This therapy could increase the proliferation of hepatocytes in liver injury, and preserved hepatocyte cells from necrosis on liver transplantation rats model.[12] This therapy also reported having a hepatoprotective effect in hepatocellular necrosis which was caused by excessive administration of acetaminophen in rats model.[18] Hyperbaric oxygen therapy could increase the level of dissolved oxygen transported to the tissues systemically,[14] moreover, oxygen transported to the injured liver will also increase.

Serum albumin is the most substantial plasma protein in the body, about 50% of the total protein, which is mainly synthesized in the liver. Albumin acts as the main modulator in the distribution of fluids throughout the body's space. Some of the albumin functions are maintaining colloid oncotic pressure in plasma and interstitial fluid, responsible for water retention, as an antioxidant and anti-inflammatory, and also in endothelial protection.[8,15] Treatment using albumin, is currently become one of the choices in the treatment of liver disease, especially in liver cirrhosis.[8]

Based on the results of this study, serum albumin levels of oral candidiasis immunosuppressed rats without hyperbaric oxygen therapy (G2) showed a decreased when compared with healthy rats group (G1) and oral candidiasis immunosuppressed rats treated with hyperbaric oxygen 2,4 ATA 3x30 minutes/day for five days (G3). The decrease in serum albumin levels was likely because of enhanced leakage in the outside vascular system.[17] It also indicates a disruption in liver hepatocytes function, as hepatocytes are an important part of the liver that is responsible for metabolizing drugs.[4] Continuous use of immunosuppressant drugs could resulting in hepatocytes to work harder, which then caused injury and reduced its function.[5] A previous study using dexamethasone as immunosuppressive drugs showed an increase in serum ALT and AST levels. The elevation of ALT and AST levels could show the damage of the hepatocellular liver.[16] Hepatocytes are also being the major part in albumin synthesis, the integrity of these cells will effect on albumin production.[17] This was similar to a previous study that showed a decreased in the amount of albumin caused by a reduced in production by hepatocytes.[19]

The impact of hyperbaric oxygen therapy on serum albumin has never been explained in prior studies.[14] In this study, the increased serum albumin level in G3 group was likely due to the antioxidant function possessed by albumin. Albumin becomes primary circulating antioxidant in the body, main extracellular source of thiols and reduced sulfhydryl group, which then act as scavengers of reactive oxygen and nitrogen species.[15,19] Albumin also known can decrease malondialdehyde levels, increased catalase activity, and glutathione levels significantly, therefore diminished reactive free radicals lead to oxidative damage, protected the tissues from highly reactive hydroxyl radicals, and impaired lipid peroxidation.[17,19] Increased oxygen level derived from oxygen-based therapy will be accompanied by increased production of its product, reactive oxygen and nitrogen species, which has a role in the pathogenesis of the disease.[11,19] However, oxygen therapy given at pressure 2,4 ATA will produce reactive oxygen species as a signaling molecule, in relevant levels for inducing antioxidants activity which can then accelerate the healing process of injured tissues, also will give benefits to regeneration of hepatocyte cells.[12] Hyperbaric oxygen therapy not only able to increase tissue oxygen level, but also could elevate growth factors and reduce inflammatory cytokines.[14] Reactive oxygen species derived from cyclic periods of hyperbaric and normoxic oxygen will affect the release of multiple growth factors by transducing signaling pathways, including those that lead to angiogenesis.[20] Giving hyperbaric oxygen therapy will modulate Nitric Oxide (NO) to enhance expression of multiple growth factors such as Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF) which able to trigger neovascularization and angiogenesis for healing injured tissue.[20, 21] Hyperbaric oxygen therapy was able to reduce the inflammatory cytokines IL-1, IL-6, and TNF-α.[22] This was in line with the function of albumin which was capable of binding to inflammatory mediators, thus inhibited inflammatory signaling pathway and prevent inflammation in injured tissue. The previous study showed that albumin becomes an effective modulator of the innate immune system that could provide benefits in administration of acute-on-chronic liver failure.[23]

Based on the result in this study, it can be concluded that serum albumin levels of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen 2,4 ATA 3x30 minutes/ day with 5 minutes interval breathing in normal air, given for five days,

shows the significant enhancing levels compared to those group without therapy. Further study can be designed to evaluate the number of hepatocytes and other biochemistry parameters such as malondialdehyde, catalase enzim, and glutathione, to find out the hepatocellular liver function in oral candidiasis immunosuppressed rats model.

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