Full Length Research Paper

The effect of hyperbaric oxygen to the growth of Mucormycosis agent *Rhizopus oryzae* strain CBS 110.17 (An in vitro preliminary study)

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Mucormycosis is one of the most rapidly progressing and lethal form of fungal infection in humans, regarded as a fatal infection which usually begins in the nose and paranasal sinuses caused by *Rhizopus oryzae*. This organism is frequently found to colonize the oral mucosa and Rhinomaxillary or rhinocerebral form is the common type. The fungus invades the arteries leading to thrombosis that subsequently causes necrosis of hard and soft tissue. Oral manifestations are often the early signs after dental extractions as an invasive portal. One potential tool for treating these infections is hyperbaric oxygen (HBO). HBO therapy was utilized in a case of mucormycosis of the maxilla. HBO suppresses fungal growth in vitro and has theoretical value in treating mucormycosis in human because it reduces the tissue hypoxia and acidosis that accompany vascular invasion by the fungus. The aim of this study was to examine the effect of hyperbaric oxygen to the growth of mucormycosis agent *Rhizopus Oryzae* strain CBS 110.17 after fourteen times treatments with hyperbaric oxygen in macroscopic and microscopic aspects. Eight plates of sabouraud agar media was cultured by 20 µl of $10^6$ CFU/mL *Rhizopus oryzae* and incubated for 72 hours at 37°C. The plates were then divided into 2 groups as control and treatment plate, where each consisting of 4 plates. The control group were remain untreated and the treatment group were treated with hyperbaric oxygen (HBO) twice a day for 7 days, 2,4 ATA 3x30 minutes. The growth colony after 72 hours incubation appeared to be as black filamentous colony on the surface of sabouraud agar. After the treatment with HBO, the colonies were changed lighter compared with the control group. Microscopic examination showed the damaged of the fungal morphology on the treatment group. This research analysed descriptively in macroscopic and microscopic aspects. Hyperbaric oxygen affected the colony characteristic of mucormycosis agent *Rhizopus Oryzae* strain CBS 110.17.

**Key words:** *Rhizopus oryzae*, Hyperbaric Oxygen.

INTRODUCTION

Mucormycosis is the common name to several different diseases caused by fungi of the order Mucorales (Sugar, 2005). Mucormycosis (Zygomycosis) is caused by several fungal species in the order Mucorales which commonly found in soil and among decaying vegetation (Berrylin and Ferguson, 2000). Mucormycosis is one of the most rapidly progressing and lethal form of fungal infection in humans, regarded as a fatal infection which usually begins in the nose and paranasal sinuses caused by *Rhizopus oryzae*. These organisms are frequently found to colonize the oral mucosa and Rhinomaxillary or rhinocerebral form is the common type (Mohanty et al., 2012). The fungus invades the arteries leading to thrombosis that subsequently causes necrosis of hard and soft tissue. Oral manifestations are often the early signs after dental extraction as an invasive portal (Auluck, 2007). One potential tool for treating these infections is hyperbaric oxygen (Segal et al., 2007). HBO therapy was utilized in a case of mucormycosis of the maxilla. HBO suppresses fungal growth in vitro and has theoretical value in treating mucormycosis in human because it reduces the tissue hypoxia and acidosis that accompany...
vascular invasion by the fungus (Ferguson et al., 1988). The aim of this study was to examine the effect of hyperbaric oxygen to the growth of mucormycosis agent Rhizopus Oryzae strain CBS 110.17 after fourteen times treatments with hyperbaric oxygen in macroscopic and microscopic aspects.

**MATERIALS AND METHODS**

Eight plates of sabouraud agar media was cultured by 20 µl of $10^6$ CFU/mL Rhizopus oryzae and incubated for 72 hours at 37°C. The plates were then divided into 2 groups as control and treatment plate, where each consisting of 4 plates. The control group were remain untreated and the treatment group were treated with hyperbaric oxygen (HBO) twice a day for 7 days, 2,4 ATA 3x30 minutes.

**RESULTS**

The results of 24 hours, 48 and 72 hours incubated of Rhizopus oryzae strain CBS 110.17 in $10^6$ CFU/ml and the control group compared to the treatment group after Hyperbaric oxygen 2.4 ATA 3x30 minutes for 7 days, twice a day (figure 1,2 and 3). This research analyzed...
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**DISCUSSION**

Rhizopus oryzae is a zygomycete filamentous fungus, well-known as saprobe ubiquitous in soil and as a

**Microscopic image of Rhizopus oryzae after HBO treatments from the previous studies**

The growth colony appears in macroscopic and microscopic aspects.

**Figure 3.** *Rhizopus oryzae* Strain CBS 110.17 : After 72 hours Incubation

**Plate 1.** After Hyperbaric Oxygen Treatments.
pathogenic/spoilage fungus, causing Rhizopus rot and mucormycoses (Battaglia et al. 2011). Mucormycosis (Zygomycosis, Phycomycosis) is an acute opportunistic infection caused by saprophytic fungus. Rhizopus is predominant pathogen accounting for 90% of the cases of rhinocerebral mucormycosis. This microbe maybe cultured from the oral cavity, nasal passage, throat and stool of healthy patients without clinical signs of infections (Madan, 2013). Rhizopus oryzae is predominant pathogen and accounts for 60% of all forms and 90% of rhinocerebral cases (Ammari et al., 2008).

In the classification by Schipper and Stalpers (1984), Rhizopus oryzae was recognized as a species with intermediate morphology and physiological characters. They investigated 47 strains from 21 species and classified all of them as Rhizopus oryzae because they...
After 72 hours incubation

After HBO treatments

Fundamentally were indistinguishable. In Abe (2007), the former name of Rhizopus oryzae CBS 110.17 is Rhizopus maydis, rDNA ITS (AB181303), idhB (AB281556), EF-1α (AB281572), act-1 (AB281498) (Abe et al., 2007). From Fungal databases nomenclature and species bank, Rhizopus oryzae CBS 110.17 came from Switzerland, isolated by A. Lendner, redet H. Zycha (1935), redet MAA Schipper (1984) and taxon name: Rhizopus Maydis (MycoBank in English, 2014). This organism is mucormycosis agent (Uniprot, 2014)

Invasive fungal infections by Mucorales species are lethal infections in immune compromised patients. For these infections a multimodal approach is required. One potential tool for treating these infections is hyperbaric oxygen (Segal et al., 2007). The use of hyperbaric oxygen as adjunctive treatment for Zygomycoses has been reported since 1970s and hyperbaric with aggressive surgical debridement, amphotericin B therapy, control of any underlying predisposing conditions by aiding neovascularization and subsequent healing (Mohanty et al., 2012). HBO therapy has also been used to treat mucormycosis (Garlapati et al., 2014). HBO therapy has been used in an attempt to control the infection. Experts suggest that HBO may exercise fungistatic activity by reducing tissue hypoxia and acidosis (Yen et al., 2011)

Pressurized hyperoxygenation (i.e. exposure to oxygen at high ambient pressures) greatly increases the oxygen transport capacity in blood and the oxygen pressure gradient across capillary membranes, thereby augmenting tissue oxygenation. This is believed to be beneficial in situations of hypoperfusion and anaerobism,
and is supported by experimental data that have demonstrated direct antimicrobial activity, restoration or enhancement of cellular defenses, synergistic effects with antimicrobials and restoration or augmentation of tissue repair (Tragiaennidis and Groll, 2009). HBO can relieve tissue hypoxia, restore oxygen necessary for the granulocyte respiratory burst, restore normal fibroblast function, relieve tissue lactic acidosis and provide oxygen for the oxidative mechanism of Amphotericin B (Barratt et al., 2001). Price and Stevents (1980) had reported about HBO therapy was utilized in a case of fulminant mucormycosis of the maxilla, orbit and temporal bone and the patient had refused radical surgery and death seemed imminent in spite of aggressive medical management of diabetic ketoacidosis, amphotericin B and wide surgical drainage of the maxillary and ethmoid sinus with orbital decompression. Hyperbaric oxygen was instituted on the following theoretical premises: 1. It would provide oxygenation of tissues distal to occluded arteries, thereby increasing local survival and decreasing acidosis. 2. Resultant lessening of acidosis would slow or inhibit rapid growth of the organism, and 3. Oxygen in sufficient concentration is fungicidal. The rapid progress of the mucormycosis was arrested. Tissue cultures prior to the hyperbaric oxygen treatment produced heavy growth of Rhizopus and tissues cultured after therapy grew only bacterial contaminants (Price and Stevents, 1980). Usually early diagnosis of mucormycosis infection is not possible and difficult because of the following reasons: 1. Late presentation of associated symptoms like pain, fever, as fungal infections does not general exuberant inflammation, 2. Extensive tissue and bone necrosis will lead us to think first in terms of osteomyelitis unless proved by culture or failure of antibiotic therapy, 3. Cultures of fungus take approximately three weeks and confirmatory diagnosis is delayed. Hence histopathology of scrape biopsy or tissue biopsy can be promising in early detection of fungi (Ahamed, 2014). With respect to the delay in initiating treatment, it appears to be that HBO was initiated later than AMB and surgery. This delay seems to indicate that HBO was used as the last sequence in most patients (Covarrubias et al., 2002).

In this research we cultured 20 µl 10⁶ CFU/ml Rhizopus oryzae Strain CBS 110.17 in saboraud agar and were treated with hyperbaric oxygen (HBO) twice a day for 7 days, 2,4 ATA 3x30 minutes (Barratt et al., 2001) and the incubator temperature was 37° C (Schaffner et all, 1986). The colony 10⁶ Rhizopus oryzae (Mucormycosis agent) strain CBS 110.17 grew after 72 hours incubation appeared to be as black filamentous colony on the surface of saboraud agar. After HBO treatments, the colony changed lighter than the control group.

CONCLUSION

Hyperbaric oxygen affected the colony characteristic of mucormycosis agent Rhizopus oryzae strain CBS 110.17. In the future maybe HBO therapy can be used as initial treatment for mucormycosis because of the rapidly progressing, lethal, fatal, fulminant and acute infection beside anti-fungal therapy and surgery.
REFERENCE


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