

# The Effect of Hyperbaric Oxygen to the Growth of *Aspergillus Brasilliensis* / *Aspergillus Niger* Strain ATCC® 16404™

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## ABSTRACT

**Objectives:** Aspergillosis is one of the fastest growing and deadly fungal infections in humans. Rhinomaxillary or rhinocerebral types are oral manifestations that often occur first after tooth extraction. Hyperbaric oxygen (HBO) is one of the potential therapies for this infection. The aim of this study was to examine the growth of *Aspergillus niger* strain ATCC®16404™ after fourteen treatments with hyperbaric oxygen in macroscopic and microscopic aspects.

**Materials and Methods:** Four plates of sabouraud agar were cultured with 20 µl 106 CFU / mL of *Aspergillus niger* strain ATCC®16404™ and incubated for 72 hours at 37°C. The plates were then divided into 2 groups as control and treatment plates, each of which consisted of 2 plates. The control group remained untreated and the treatment group treated with HBO O2 100% 14 times (twice daily for 7 days) 2.4 ATA 3 x 30 minutes.

**Results:** Growth colonies after 72 hours of incubation appeared as black filament colonies on the surface of the Sabouraud agar. After treatment with HBO, the filament colony changed color brighter compared to the control group. Microscopic examination showed fungal morphological damage in the treatment group. This research was analyzed descriptively in macroscopic and microscopic aspects.

**Conclusion:** Hyperbaric oxygen affects the characteristics and inhibits the growth of the *Aspergillus niger* strain ATCC®16404™

## KEY WORDS

*Aspergillus niger* strain ATCC®16404™, hyperbaric oxygen

## INTRODUCTION

Aspergillosis is a fungal disease that can be non-invasive and invasive. Increased mycotic infections can be caused by immunocompromised conditions (immune deficiency diseases such as diabetes) and immunosuppressive agents that support fungal growth (long-term treatment with antibiotics and cortisone, radio and chemotherapy)<sup>1,2)</sup>. In India, the prevalence of *Aspergillus* infection is 54.4% of all mycotic infections. This is the most opportunistic infection that affects the maxillofacial area, then candidiasis and mucormycosis. Oral aspergillosis is dominant in men between the ages of 5-78 years<sup>3)</sup>.

*Aspergillus* fungi are widespread in nature and usually do not cause invasive disease in healthy individuals with good immunity. *Aspergillus fumigatus* is one of the *aspergillus* species that most often causes disease in individuals who have immune disorders. Often occurs in horticultural workers who breathe peat dust. The most common clinical presentation is lung and rhinosinusinal infections<sup>4,6)</sup>. *Aspergillus flavus* is the second most common agent of aspergillosis. *Aspergillus niger* can cause opportunistic infections, it is less likely to cause human disease than some other *Aspergillus* species, but if large amounts of spores are inhaled then aspergillosis can occur<sup>6)</sup>.

In immunocompromised patients, there is gingival ulceration in the gingival sulcus or marginal gingiva, in the mucosa and soft tissue dif-

fuse diffuse swelling with gray tones<sup>7)</sup>. Agustine et al (2012) reported a 60-year-old man, farmer, heavy smoker, hypertension, have a history of diabetes for 10 years with poor oral hygiene. He had diffuse swelling in the entire palate and his ulcers were covered with white fluid. On histological examination, necrotic bone and *aspergillus niger* appear with long, branched hyphae. The patient had manifestations in the anterior maxillary gingiva and the entire palatal mucosa. He had surgery and antifungal therapy without hyperbaric oxygen therapy<sup>8)</sup>.

The current standard of care involves intravenous antifungal agents, surgical debridement and correction of predisposing factors. Mortality reported as 77% is leukemia and neutropenia, and 66% for a subset of rhinosinusinal invasion. In addition, most clinics show that surgical debridement of *Aspergillus* sinusitis increases mortality among neutropenic patients due to large postoperative bleeding. Therefore, new non-invasive therapeutic devices are needed. Decreased oxygen levels cause tissue hypoxia in patients infected with Aspergillosis because this fungus attacks blood vessels, causing blockages and thrombosis.

Hyperbaric oxygen therapy (HBO) is a safe therapy by breathing 100% oxygen in an air chamber at a pressure of 2.4 atmosphere absolute (ATA). This can increase the partial pressure of oxygen in normal and infected tissue, providing the oxygen needed to inhibit the growth of organisms including bacteria and fungi. In vitro research suggests the role and benefits of HBO in the management of invasive fungi<sup>9,10)</sup>. However, the literature revealed only two case reports of invasive asper-

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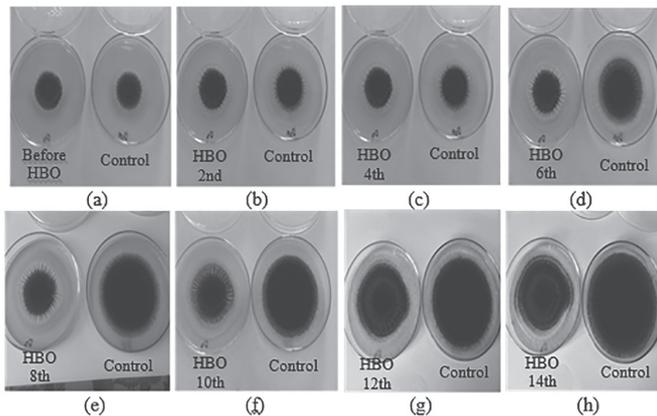
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**Figure 1: Results of the growth of *Aspergillus niger* strain AATCC®16404™ in plate 1 before HBO (a), after HBO 2nd (b), HBO 4th (c), HBO 6th (d), HBO 8th (e), HBO 10th (f), HBO 12th (g) and HBO 14th (h)**

gilliosis in which HBO was used as adjunctive therapy<sup>9</sup>. Therefore, this study can prove the potential role of HBO in the management of invasive Aspergilliosis.

The aim of this study was to examine to the growth of Aspergilliosis agent, *Aspergillus niger* strain ATCC®16404™ after fourteen times treatments with hyperbaric oxygen in macroscopic and microscopic aspects.

## MATERIALS AND METHODS

Eight plates of sabouraud agar agar were cultured with 20 µl 106 CFU / mL of *Aspergillus niger* strain ATCC®16404™ and incubated for 72 hours at 37°C. Then the plates were divided into 2 groups as control and treatment plates, each of which consisted of 2 plates. The control group remained untreated and the treatment group was treated with hyperbaric oxygen (HBO) 14 times, twice a day for 7 days using a 100% oxygen pressure of 2.4 ATA for 3 x 30 minutes.

## RESULTS

The results were obtained after 24 hours, 48 and 72 hours incubated of in 106 CFU/ml *Aspergillus niger* strain ATCC®16404™. The control group was compared with the treatment group after HBO O<sub>2</sub> 100% 14 times 2,4 ATA 3 x 30 minutes for 7 days (twice a day). This research was analyzed descriptively in macroscopic and microscopic aspects.

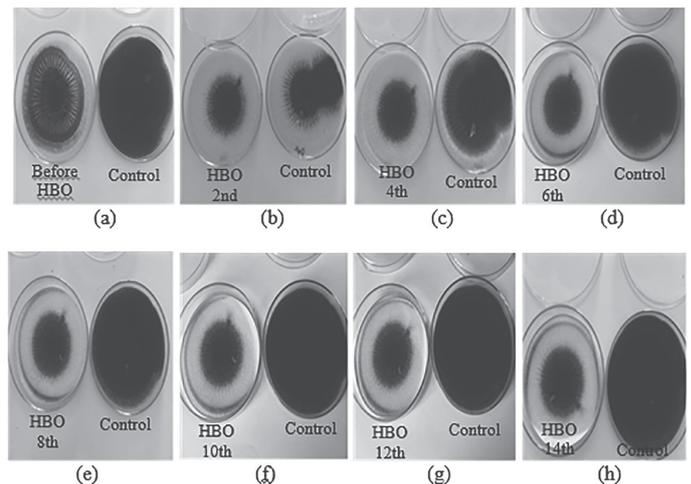
### Macroscopic Image

These are shown in Fig 1 & Fig 2.

## DISCUSSION

*Aspergillus niger* with widespread spores, is everywhere in nature and is commonly found as saprophyte that grows on dead leaves, seeds, compost piles, and other rotten vegetation. *Aspergillus niger* has been reclassified in *Aspergillus brasiliensis* (refer to publication by Varga et al)<sup>11</sup>. This requires an update to US Pharmacopoeia which normally uses this strain throughout the pharmaceutical industry.

Case fatality rates are 58% - 67%. This shows that any invasive aspergilliosis remains a highly lethal opportunistic infection despite the most recent antifungal therapy and improved management of the underlying disease<sup>12</sup>. Possible complications are cavernous sinus thrombosis, perforation of the palate, expansion of the orbitals and cranial and severe facial defects<sup>8</sup>. Aspergilliosis of the paranasal sinuses has been classified into four types: allergic, non-invasive, invasive, and fulminant. Immunocompromised patients have a risk of suffering from fulminant invasive aspergilliosis. The most frequent aspergillus infection in humans is in the lung tissue, followed by the liver, spleen, bones, meninges, and paranasal sinuses. In several reports, the coexistence of fungal infections



**Figure 2: Results of the growth of *Aspergillus niger* strain ATCC®16404™ in plate 2 before HBO (a), after HBO 2nd (b), HBO 4th (c), HBO 6th (d), HBO 8th (e), HBO 10th (f), HBO 12th (g) and HBO 14th (h)**

and malignancies has been noted in the brain and thoracic cavity<sup>13</sup>.

The etiopathogenesis of maxillary aspergilliosis has been debated and there are 3 theories: the theory of odontogenic, aerogenic and mixed origin. Odontogenic theory states the initial colonization of the maxillary sinus through iatrogenic oral-antral communication. Zinc oxide can be found in endodontic sealers thereby paralyzing epithelial cilia, causing edema and soft tissue hyperemia, affecting Scheiderian epithelial function (sinus membrane). This supports the accumulation of fungal waste and undermines the elimination of spores<sup>14</sup>. Aerogenic theory states that aspergillus growth is caused by inhaling high amounts of spores over a long time. This is also called the form of semi-invasive fungal sinusitis. Mixed theory is based on the ubiquitous nature of aspergillus spores. They can be inhaled at any time and normally present as saprophytes in the maxillary sinus. *Aspergillus* growth can be caused by poorly ventilated sinuses, pre-existing sinusitis or foreign bodies in the sinuses<sup>15</sup>.

Oral manifestation of invasive aspergilliosis caused by *Aspergillus niger* found as diffuse swelling of the entire palate, the palatal mucosa was edematous and swollen in a 60 year old male<sup>9</sup> and in a 19 year old male patient presented with fever, rhinitis and progressive pain behind left eye. He had undergone a maxillary tooth extraction and the alveolar wound did not heal due to osteomyelitis<sup>16</sup>. The cases was treated with antifungal therapy and aggressive surgery but mortality rate with the current therapy has remained unacceptably high. In vitro data along with a few case reports have suggested a potential benefit of hyperbaric oxygen (HBO)<sup>9</sup>.

In both aspergilliosis and mucormycosis, hyperbaric oxygen therapy has also been suggested for combination therapy especially in case of cerebral abscess. The important effects of hyperbaric oxygen therapy are the increase of free oxygen radicals which inhibit bacterial metabolism, the direct killing of anaerobes and the stimulation of the oxygen-dependent peroxidase system which enables leukocytes to kill microorganism<sup>17</sup>. In a retrospective clinical series, invasive aspergilliosis treated with adjunctive HBO seems to have improved the patient's condition. This observation was supported by previous in vitro studies where HBO clinically showed a fungistatic effect on various fungal species including aspergillus<sup>9</sup>.

In this in vitro preliminary study, we found that *Aspergillus niger* who had been treated with hyperbaric oxygen had a different size and color compared to the control group. *Aspergillus niger* grew faster in the control group and had a darker color than the treatment group. This means that HBO affects colony activity and inhibits fungal growth but the mechanism of HBO as an antifungal *Aspergillus niger* requires further research.

## CONCLUSION

Hyperbaric oxygen affected the colony characteristic and inhibits

fungal growth of aspergillosis agent *Aspergillus niger* strain ATCC®16404™.

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