# Averting Crippling Fungal Super-Infection in Era of Viral Pandemic: A Case-Control Retrospective Evidence-Based Study

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**Abstract**

The second wave of COVID-19 has affected India substantially, with the highest number of daily cases reaching 0.4 million in a single day. COVID-19 infection, its treatment, resultant immune suppression, and pre-existing co-morbidities have made patients vulnerable to secondary infections including mucormycosis. Nasal irrigation is an ancient and well-practiced treatment for nasal and sinus pathologies. It has been proved that Mucorales live as commensals in nasal epithelium and a drop in immunity leads to its super-infection. This practice of nasal douching reduces its survival and washes out the spores from the nasal cavity.

The study aims to prove that practicing Nasal Irrigation with prescribed medications reduces the incidence and severity of Mucormycosis.

110 patients who reported with Post-Covid sinusitis were selected for the study. History of Covid-19 infection was taken. Pre-treatment KOH and culture were advised. Also, pre-treatment radiological analysis was done to assess the severity of involvement. According to the guidelines, antibiotic, antifungal, decongestant, anti-inflammatory, and antacid drugs were prescribed. Along with this, nasal irrigation of buffered saline solution, povidone-iodine, and 2% acetic acid was prescribed to alleviate the symptoms or to prevent them all together. Post-treatment culture and radiological analysis were carried out if symptoms persisted.

Collected data underwent Chi-square test. The P-value of the study was kept at less than 0.05 according to the sample size.

The study revealed a significant decrease in the incidence and severity of symptoms of mucormycosis.

This study concludes that Nasal Irrigation has an important role in the prevention of crippling Mucormycosis in Post-Covid patients with initial symptoms of sinusitis.

**Clinical article (J Int Dent Med Res 2022; 15(1): 178-183)**

**Keywords:** Mucormycosis, Nasal Lavage, Sinusitis.

**Received date:** 03 November 2021 **Accept date:** 28 December 2021

## Introduction

The global Corona-virus pandemic which is a global health concern has infected 250 million people internationally and 34 million people in India alone till date[1,2] Caused by coronavirus-2 (SARS-CoV-2), the disease may progress to acute respiratory distress syndrome (ARDS), a condition that increases the susceptibility of pulmonary fungal co-infections like Mucormycosis.[3,4] Mucormycosis is an angio-invasive fungal infection of the order Mucorales. The incidence of mucormycosis has risen more rapidly during the second wave compared with the first wave of COVID-19 in India with at least 14872 cases. The state of Gujarat alone contributed to the highest number of cases, with at least 3726 cases. Gujarat declared it as an epidemic and notifiable disease to national health authorities.[5] Although no official figures about mucormycosis in COVID-19 patients were released by Union Health Ministry during the first wave, India contributed to 71% of the global cases of mucormycosis in patients with COVID-19 based on published literature from December-2019 to April-2021[6]. This may be because India

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has the second-largest number of adults aged 20-79 years with Diabetes Mellitus (DM). DM is the single most common risk factor for mucormycosis in India, being reported in over 50% of cases of Mucormycosis.[7] Other possible risk factors may be over usage of steroids, post-COVID immunosuppressive states, unhygienic water in the humidifier, use of industrial oxygen, contaminated oxygen tubing, high ferritin and iron levels in the blood, and excessive use of zinc.

Infection of Rhino-Orbital-Cerebral Mucormycosis (ROCM) type, the most commonly manifested form of mucormycosis begins with inhalation of spores that allows the fungus to spread to the paranasal sinuses. The infection can then rapidly extend to the palate, the sphenoid sinuses, the orbits, and the cavernous sinuses, and eventually to the brain. Hence, the main initial presenting symptom is Unilateral or Bilateral sinusitis with facial pain or heaviness. Rapid diagnosis of ROCM and prompt treatment are crucial for the prevention of extension to orbital and cerebral tissues.[8] Microbiological diagnoses, control of the underlying systemic condition, and antimicrobial therapy with debridement of necrotic tissue have remained the mainstay of management of rhino-orbital mucormycosis over the years. A delay of even six days in initiating treatment doubles the 30-day mortality from 35% to 66%.[16]

Fungal infections can thrive well mainly because the biochemistry of fungal species is comparable to that of a human host. This makes synthesizing a drug to combat fungal infection without affecting human host very difficult. It is henceforth why most of the currently available antifungal drugs are known to cause side effects and the need for alternative regimens became the need of the hour.[9] Also, topical nasal and paranasal sinus irrigation with medications reduces the incidence and severity of symptoms which is not included in any mucormycosis treatment protocol.

Nasal irrigation is an ancient and well-practiced treatment for nasal and sinus pathologies. It has its roots in Ayurveda, where it was termed as “Jala-neti”, which in Sanskrit literally means “nasal cleansing”[10] This technique uses gravity to flush out allergens, pathogens, and debris along with the irrigating solution through the nasal cavities. This age-old technique holds the potential to reduce the severity of symptoms of Mucormycosis and prevent it.

So the null hypothesis is that practicing Jal-Neti with prescribed medications does not have any role in the incidence and severity of Mucormycosis.

## Materials and methods

The retrospective, single-centre, single-blinded Case-control study was carried out at a private centre located in Junagadh, Gujarat. The study protocol followed the CONSORT guidelines (http://www.consort-statement.org) perspicaciously. Inclusion criteria were all the post-COVID patients irrespective of severity with the initial symptom of sinusitis. All children less than 10 years who are unable to follow instructions and who cannot tell their symptoms were excluded from the study.

110 patients who reported with Post-COVID sinusitis were selected for the study and informed consent was taken. History of COVID infection was taken. Pre-treatment KOH and culture were advised. Also, pre-treatment radiological analysis was done to assess the severity of involvement. According to the guidelines, antibiotic, antifungal, decongestant, anti-inflammatory, and antacid drugs were prescribed.

Along with this, nasal irrigation was prescribed to alleviate the symptoms or to prevent them all together. For the first step, patients were advised buffered saline irrigation. For this, 3 teaspoons of salt with 1 teaspoon of baking soda had to be mixed and stored. Now 1 teaspoon of this mixture was to be mixed in 1 cup of lukewarm boiled or distilled water. The patients were advised to use less to make less concentrated salt solution if burning or stinging is experienced. Patients were using a soft rubber ear bulb syringe, infant nasal bulb, or commercial nasal saline rinse (NasoClear wash). They were instructed to draw up 1 cup of saline solution and tilt their head down over the sink (Mecca position) and squeeze half of the solution gently into the right nostril and breathe through the mouth. In a few seconds, the solution would come out of the left nostril, and then patients were told to repeat the process on the other side. For the second step, 1.25% povidone-iodine nasal irrigation was advised similarly.

For the third step, 2% acetic acid irrigation was advised. Commercially available white

vinegar is 4% acetic acid which was told to be diluted with distilled or boiled water in a 1:1 ratio to attain the desired concentration of 2%.

This process of nasal irrigation was advised for 15 days and then patients were called for follow-up.

In a follow-up visit, other symptoms of mucormycosis were assessed and further investigations like post-treatment culture and radiologic analysis were done. All the data was collected and analyzed using the Chi-square test.

## Results

Out of total 126 patients with Post-COVID sinusitis, 83 were male and 43 were female. The mean onset of symptoms of mucormycosis was 31.64 days. (Table 1) 3 patients were active cases of COVID-19 and 4 patients had no prior history of COVID-19. 74 patients had a history of diabetes mellitus which constitutes 58.7% of patients. 88(69.8%) patients were hospitalized, 67(53.17%) patients were on oxygen support and 86 (68.25%) patients were on steroids. (Table 2). Initial symptoms with which patients reported ranged from facial burning, heaviness, sinusitis to diplopia, proptosis, sinus openings and pigmentation in the oral cavity, crusting in nostril and middle meatus. Majority of patients had an initial symptom of sinusitis along with other symptoms. Endoscopy was done in 36(28.5%) patients where, crusting, debris, scabbing, granulation, discoloured mucosa (either darkened or pale), decreased bleeding and insensate mucosa were found. In pre-treatment KOH, 72 (57.17%) patients reported with non-septate or pauci-septate ribbon-shaped hyphae. In pre-treatment culture, 56 patients had Rhizomucor, 11 patients had Rhizopus oryzae, 7 patients with Aspergillus Flavus, and 3 patients with Aspergillus Niger, and the rest were other species. (Table 3) In 64 patients, MRI showed Mucosal thickening of sinuses Changes in Fat Planes, Erosion, and thinning of bones. 11 patients on CT PNS showed pan-sinusitis with mild to invasive bone erosion. The average numbers of consultations were 2.54, the maximum being 7. In 16 patients (12.8%), symptoms were not relieved and post-treatment KOH and culture showed scanty fungus. They were referred to higher centres for surgery or biopsy. (Table 4, Graph 1)

| Variable | N | Minimum | Maximum | Mean |
|---|---|---|---|---|
| Age | 126 | 7.00 | 70.00 | 47.8016 |
| *-Males* | 83 | 7.00 | 70.00 | 49.6747 |
| *-Females* | 43 | 18.00 | 65.00 | 44.1860 |
| Onset after COVID (days) | 12 | 5.00 | 300.00 | 31.6417 |

**Table 1.** Demographic data of patients.

| Variable | | Frequency | Percentage |
|---|---|---|---|
| **Medical History** | Diabetes Mellitus | 74 | 58.7 |
| | Hypertension | 26 | 20.6 |
| | Ischemic Heart Disease | 5 | 3.9 |
| | No Medical History | 21 | 16.66 |
| **Hospitalized** | | 88 | 69.8 |
| **Oxygen Support** | | 67 | 53.2 |
| **Steroid Therapy** | | 86 | 68.25 |

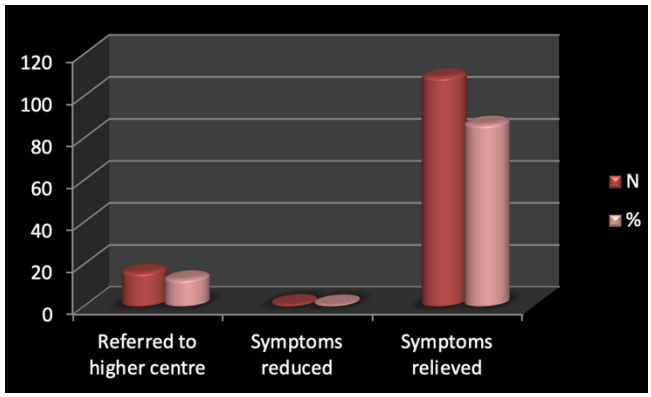
**Table 2.** Onset of COVID.

| Variable | Items | Frequency | Percent |
|---|---|---|---|
| Endoscopy | Yes | 36 | 28.6 |
| Pre-treatment | No Hyphae | 46 | 36.5 |
| KOH | Non-septate ribbon-like hyphae | 52 | 41.2 |
| | Pauci-septate hyphae | 16 | 12.6 |
| | Septate hyphae | 4 | 3.2 |
| Pre-treatment | Aspergillus Flavus | 7 | 5.5 |
| culture | Aspergillus Niger | 3 | 2.3 |
| | Rhizomucor | 51 | 40.47 |
| | Rhizopus oryzae | 11 | 8.7 |
| | No Fungus | | 42.8 |

**Table 3.** Pre-treatment Investigation.

| | N | % |
|---|---|---|
| **Referred to higher center** | 16 | 12.8 |
| **Symptoms reduced** | 1 | 0.8 |
| **Symptoms relieved** | 109 | 86.4 |

**Table 4.** Outcome of study.




**Graph 1.** Outcome of study.

## Discussion

The second wave of COVID-19 has affected India substantially, with the highest number of daily cases reaching 0.4 million in a single day. COVID-19 infection, its treatment, resultant immune suppression, and pre-existing co-morbidities have made patients vulnerable to

secondary infections including mucormycosis. Patients with COVID-19 are more prone to fungal infection because of the compromised immune system with lowered CD4+ and CD8+ lymphocytes, associated comorbidities such as diabetes mellitus which potentiates both the conditions, compromised pulmonary functions, and the use of immunosuppressive therapy for the management in moderate to severe cases.[11]

The super-infections are also more likely in patients with severe COVID-19 disease and in those requiring intensive care unit admission or mechanical ventilation. In a review, Song et al. noted that fungal infections are more likely to develop during the middle and later stages of COVID-19 infection[12] The mortality rate is also higher (53% with vs 31% without invasive fungal infection) amongst the patients of COVID-19 with secondary fungal infection.[9]

This case-control study was undertaken at a private centre in Junagadh during the peak of the second surge of COVID-19 in India during May-2020 to July-2020.

65% of the reported cases were in males. The reason for a higher prevalence of Mucormycosis infections among males is unclear. Estrogen plays a protective role against fungal infection. The potential role of estrogen in Mucormycosis infection has not yet been explored.[13] The mean onset of symptoms of Mucormycosis after COVID-19 found in this study is 31.64 days. It is reported that ROCM mucormycosis may develop as late as 3–42 days post-COVID- 19 diagnosis or those who have recovered from the infection.[14,15]

The most common systemic risk factor was uncontrolled diabetes (58.7%). COVID-19 positivity and concurrent steroid use further decreased the immunity in 88% of patients. This correlation has been reported in the earlier studies of the COVID-19.[16,17,18] 68% of the patients were on oxygen support. It has been postulated that industrial oxygen or oxygen concentrators could be contaminated with spores. But mucormycosis is an epidemic within pandemic; hence it is illogical to believe that all oxygen supplies in the country are infested with Mucorales. Also, this theory fails to explain as to why oxygen contaminated with fungal spores is causing rhino-cerebral variant and not pulmonary.

The fungus commonly resides as a commensal in the nasal mucosa. Fungal spores enter mostly via inhalation and subsequently enter the paranasal sinuses. Affected individuals usually present with acute sinusitis, fever, nasal congestion, purulent nasal discharge with crusting, and headache.[19] In 36 patients, black crusting was visible in either or both nostril and middle meatus, and endoscopy was performed according to guidelines. In 80 patients, non-septate or pauci-septate hyphae were seen, typical of Rhizopus species. Apart from that Aspergillosis flavus, Aspergillosis navi, and Rhizomucor species were also isolated in pre-treatment culture. All patients were prescribed antibiotics, antifungals, and decongestant drugs according to guidelines.

Along with that, they were prescribed nasal irrigation with medicaments. Nasal irrigation is an ancient and well-practiced treatment for nasal and sinus pathologies. It has been proved that Mucorales live as commensals in nasal epithelium and a drop in immunity may lead to its super-infection. This practice of nasal douching reduces its survival and washes out the spores from the nasal cavity. Earlier studies have shown a mechanical intervention, where it causes mucus lining softening and dislodgement, and removal of inflammatory mediators like leukotrienes and prostaglandins, irrespective of the composition of the solution used.[20]

Firstly, patients were advised buffered saline irrigation. For this, 3 teaspoons of salt with 1 teaspoon of baking soda had to be mixed and stored. Now 1 teaspoon of this mixture was to be mixed in 1 cup of lukewarm boiled or distilled water and nasal irrigation had to be done. This mixture has an alkaline pH of about 8.3 which is proved to be fungicidal.[21,22] Ramalingam et al proved with laboratory evidence that non-myeloid cells, such as epithelial cells and fibroblasts, have an innate immune mechanism, which fires into action in the presence of sodium chloride, by producing hypochlorous acid from chloride ions.[23]

Hypochlorous acid, which is the active ingredient in bleach, has a known antifungal action. Hence, by providing chloride ions in the form of nasal saline irrigation, we can supplement the action of hypochlorous acid.

Although the salt concentration of the irrigating solution is related to the composition and activity of the sinonasal secretions,[24] isotonic solutions were found to have an immediate as well as significant effect on reducing the microbial load. On contrary, hypertonic solutions

had only a marginal effect. Also, after 24 hours of nasal irrigation, concentrations of lysozymes and lactoferrins were found to be increased by 30%.[25]

Bicarbonate ions reduce the viscosity of the mucus. Bicarbonate ions also act as an exfoliating agent and help neutralize the virus by irritating the viral envelope. Sodium chloride and sodium bicarbonate do not react with each other. Both contain sodium ions, so a double replacement reaction is not possible. The chloride ion is a very weak base to de-protonate the bicarbonate ion. Hence, there is no significant acid-base reaction. Moreover, both these ions are neither strong oxidizing agents nor reducing agents. Therefore, even redox reactions do not occur. Thus, alkaline nasal irrigation is a comparatively safe method of moisturizing the nasal mucosa and facilitating the clearance of mucus, crusts, and debris.

For the second step, 1.25% povidone-iodine nasal irrigation was advised in the similar way. Povidone-iodine (Betadine) is prepared as a soluble complex of iodine bound to povidone which acts as a synthetic inert carrier. Free iodine has been shown to be microbicidal against various bacteria, fungi, and viruses, while also having an anti-inflammatory effect. Moreover, resistance to povidone-iodine has yet to be recognized owing to its broad spectrum of activity, hypersensitivity reactions are rare, and efficacy against biofilms has been demonstrated.[26]

For the third step, 2% acetic acid irrigation was advised. Commercially available white vinegar is 4% acetic acid which was told to be diluted with distilled or boiled water in a 1:1 ratio to attain the desired concentration of 2%. Acetic acid is highly effective against Mucorales, even at 0.3%. This anti-fungal effect is not evident with other acids, such as hydrochloric acid and lactic acid, which suggests that acetic acid against Mucorales spores is not exclusively evoked by low environmental pH. Acetic acid is an easily available compound that is temperature stable and non-hazardous. It may offer an effective, low-cost, preventive treatment in post-COVID patients in which fungal symptoms have just developed and not progressed.

Even though in 11% of patients' fungus was not totally eliminated but symptoms were relieved to a great extent. Further clinical trials on large scale should be done to revive nasal irrigation to alleviate symptoms of Mucormycosis when occurring in an epidemic where medical help cannot reach all.

## Conclusions

Hence, the null hypothesis is rejected and nasal douching provides hope against this debilitating fungal disease. 88% of patients had reduced symptoms and the disease did not progress further. Even though 11% of patients had to be referred for surgery or biopsy, this method alleviated their symptoms to a greater extent.

## Acknowledgements

The authors would like to thank Dr. Amirul Haque for his valuable contribution. The research received no specific grant from any funding agency.

## Declaration of Interest

The authors report no conflict of interest.

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