

## Role of Methylene Blue in COVID-19 associated Mucormycosis

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### Abstract:

Invasive fungal sinusitis is a life-threatening disease present in immunocompromised individuals with impaired neutrophilic response. Fungi in the order of Mucorales are the most commonly implicated species. <sup>(1)</sup> Once a rare disease, incidence of Mucormycosis saw a rapid increase in May 2021 in India, mostly in patients suffering from or patients who had recovered from COVID-19 infection.

To face the new epidemic of Mucormycosis in the face of an existing Pandemic of COVID-19, the healthcare system in India is coming up with newer management strategies for improvement of patient condition. One of such interventions is the introduction of methylene blue nebulization in such patients.

**Keywords:** Mucormycosis, methylene blue prophylaxis

### Introduction:

Corona virus is a single stranded RNA virus, belonging to the family Coronaviridae, causing illnesses ranging from common cold to severe acute respiratory syndrome (SARS). A novel corona virus disease COVID-19 was initially noted in Wuhan, China in 2019. It rapidly spread around the world and WHO announced it as a Pandemic in March 2020.

India saw a surge in COVID-19 cases after a period of decline, in mid-March 2021. The first case of Mucormycosis seen in a post COVID-19 infected patient was reported in Mumbai in May 2021. <sup>(2)</sup> Thereafter several cases have been reported from across the country.

Mucormycosis is an invasive fungal sinusitis, characterized by the presence of hyphal invasion of sinus tissue. There is mycotic infiltration of blood vessels, vasculitis with thrombosis causing tissue infarction. As a result, there is a black eschar formation and associated blackish nasal discharge – hence the misnomer “black fungus”. <sup>(3,4)</sup>

The condition rapidly progresses within a time course of 4 weeks and can be fatal. *Rhizopus*, *Rhizomucor* and *Mucor* are the commonly implicated species. The inability of the host to mount an immune response makes the fungus to spread rapidly, invade blood vessels and cause bony erosions. Thus, it is an opportunistic pathogen in immunocompromised individuals, but the absence of predisposing factors does not exclude the presence of mucormycosis.<sup>(5)</sup> Most of the deaths is secondary to intra-orbital and intra-cranial complications.

Methylene blue is a water-soluble thiazine dye that was synthesized in 1876. It has various uses in the field of medicine. As a dye, it is popular in Otorhinolaryngology, in the endoscopic marking of tissues for localization, to study the ciliary function test in the nose and to stain the sinus tract for excision in cases of pre-auricular sinus.

It also has a therapeutic role in malaria, methaemoglobinemia, catecholamine refractory vasoplegia, septic shock, hepatopulmonary syndrome, photodynamic therapy for cancer, etc.

The basis of its action depends on the fact that it is an inhibitor of nitric oxide synthase and guanylate cyclase.<sup>(6)</sup> A study by Ansari et al showed that the antifungal activity of methylene blue was secondary to mitochondrial dysfunction and disruption of redox and membrane homeostasis.<sup>(7)</sup> Methylene blue is found to have lower toxicity compared to antifungal drugs and can be easily eliminated from the body. Approximately 40% of methylene blue is excreted by the kidneys and majority of it is metabolized in the liver. Another study by Gilberto L. et al, has shown that methylene blue might protect the patient against COVID-19 associated hyperinflammatory action by its anti-inflammatory effects.<sup>(8)</sup> This study was carried out with an aim to study the effectiveness of methylene blue in the prophylaxis of COVID-19 associated Mucormycosis.

### **Material and methods:**

Inclusion criteria:

All COVID-19 patients admitted to McGann hospital between June 2021 to October 2021 and willing to give consent for participation in the study were included in the study.

Exclusion criteria:

- Patients with known G6PD deficiency
- Patients on anticoagulants
- Pregnant women / lactating mothers
- Patients not willing to participate in the study

Study was undertaken after obtaining approval from institutional ethical committee. Methylene blue was used as a prophylactic agent to study its effectiveness in preventing COVID-19 associated mucormycosis. In case of development of nasal stuffiness, nasal discharge, headache, eye pain, swelling of face and/or skin and mucous membrane discoloration during the course of study, patients were termed as suspected Mucormycosis cases and were subjected to KOH stain and fungal culture.

Procedure of methylene blue administration:

10 ml of methylene blue was diluted in 200 ml of distilled water. 5ml of this solution was added to each oxygen humidifier and patient was made to inhale twice daily. Precaution was taken not to use tap water or contaminated water.

All patients were followed up for a period of 6 weeks for the occurrence of mucormycosis.

Patient data was entered in Microsoft Excel software. The required data was later obtained from the excel sheet.

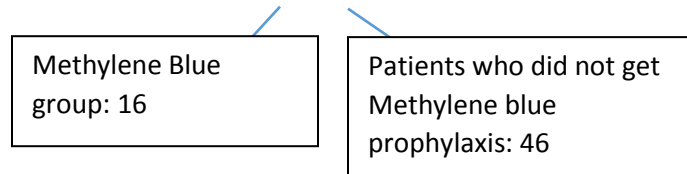
### Results:

A total of 6259 patients were admitted to the hospital in the study period. After explaining about the study in detail, 1916 patients gave consent to participate in the study.

Total no. of COVID patients admitted to the hospital: 6259

Total no. of COVID patients in whom methylene blue was administered as a prophylaxis: 1916

Total no. of COVID-19 associated mucormycosis cases in the study period: 62



The data can be put into a 2x2 contingency table to test for the significance of use of methylene blue as a prophylactic agent in COVID-19 associated mucormycosis.

Table 1: Comparison between patients given methylene blue and those not given methylene blue

<b>Methylene Blue</b>	<b>Mucormycosis Positive</b>	<b>Mucormycosis Negative</b>	<b>Total</b>
<b>Yes</b>	16	1900	1916
<b>No</b>	46	4297	4343
<b>Total</b>	62	6197	6259

On applying chi-square test, the p value is 0.408

Therefore, there was no significant difference between patients that were given methylene blue and patients who were not given methylene blue.

### Discussion:

A clinical trial by Hamidi -Alamdari et al was conducted to study the effect of reduced form of methylene blue on the improvement of oxygen saturation and respiratory rate in COVID-19 patients. They concluded that addition of methylene blue to treatment protocols significantly improved the SpO<sub>2</sub> and respiratory distress in COVID-19 patients. <sup>(9)</sup>

Mucormycosis, which was previously a rare infection seen in diabetic or immunocompromised patients in the pre-covid times became quite common in the second wave of COVID-19 because of the disease and its treatment. <sup>(10)</sup> When India saw a sudden spike in mucormycosis cases

among COVID-19 patients, the causes were thought to be the widespread use of industrial oxygen (because of crisis of medical oxygen) and humidifiers (usage of tap water instead of distilled water or mold-tainted humidifiers). Dr. Ajay Mohan Aggarwal said that a few drops of methylene blue when added in the humidifier, moisturizing jar, or concentrators through which oxygen passes would control almost all types of contamination, including mucormycosis, present in oxygen. <sup>(11)</sup>

Dr. Deepak Golwalkar reported that around 7000 patients were treated with methylene blue and none of them developed mucormycosis. He said that methylene blue could be given orally, through a nebulizer or via intravenous route. <sup>(12)</sup>

A group led by Ashwin Sankdasariya believes that methylene blue is an effective and cheap weapon against Covid and Mucormycosis. They ran six covid isolation centers and treated patients there using methylene blue, and none of the patients treated buy them got mucormycosis. <sup>(12)</sup>

In our study, not all covid pateints gave a consent to participate in the study. So, methylene blue could be administered only to 1916 covid patients. 1900 patients among them did not develop mucormycosis. Although methylene blue was not proven to be an effective prophylactic agent after application of tests of significance, no adverse effects were noted in any patients in whom methylene blue was administered.

### **Conclusion:**

Methylene blue is a commonly used dye whose other uses in medical field has been defined. Its recent use in the improvement of saturation in COVID-19 patients were shown by few studies. However, its use as a prophylactic agent in preventing mucormycosis associated with COVID-19 has not yet been proven. Although our study did not show a significant benefit from the use of methylene blue in prevention of COVID-19 associated mucormycosis, Extensive studies on a large scale are required before ruling it out as an effective prophylactic agent.

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