

Original article

## **Methylene Blue for Treatment of Hospitalized COVID-19 Patients, Randomized, Controlled, Open-Label Clinical Trial, Phase 3**

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### **Abstract**

**Background:** Methylene blue (MB) possesses all the required properties for the clinical management of COVID-19. We have coined methylene blue as an anti-hypoxemia and anti-respiratory distress agent and previously witnessed promising results in phase 1 & 2 clinical trials.

**Methods:** In the phase 3 clinical trial, the efficacy of MB has been evaluated as an adjunct therapy along with standard care protocols in the treatment of COVID-19 patients. A randomized, controlled, open-label clinical trial was conducted from 20 September through to 20 November 2020, and two hundred twenty-three hospitalized patients with confirmed severe cases of COVID-19 were recruited. Patients were randomly assigned to receive either oral MB (the reduced form: 1mg/kg T.I.D. for 2-days, followed by 1mg/kg B.I.D. for the next 12 days) along with standard care (MB-group: 106) or standard care alone (SC-group: 117). The outcomes were duration of hospital stay and mortality.

**Results:** The hospital stay, measured in days, was significantly shortened in the MB-group ( $6.2 \pm 3.1$ ) in comparison to the SC-group ( $10.6 \pm 9.2$ ,  $p < 0.001$ ), and a marginal significant decrease in mortality was seen in the MB-group (12.2%) in comparison to SC-group (21.4%,  $p = 0.07$ ).

**Conclusions:** We conclude that MB, as an adjunct therapy, can be used along with standard care protocols for the treatment of COVID-19. Larger studies in other centers are needed to confirm these findings. MB is a low-cost and FDA-approved drug for methemoglobinemia.

**Keywords:** COVID-19; Treatment; Methylene Blue; Mortality

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## Introduction

In the clinical management of COVID-19 patients, oxygen support, anti-viral agents, antibiotics, anticoagulants, immunomodulatory drugs, antioxidants and fluid therapy are applied (Shenoy et al, 2020; Perkinset al, 2020). Methylene blue (MB) possesses all the required medicinal characteristics along with anti-hypoxemia and anti-respiratory properties, making it a candidate drug for the treatment of COVID-19 (Alamdari et al, 2020;, Alamdari et al, 2020, Alamdari et al, 2021).

In our phase 1 (Alamdari et al, 2020) and 2 (Alamdari et al, 2021) clinical trials, we determined the effectiveness of MB in the treatment of COVID-19 patients and coined MB as an anti-hypoxemia drug (reduced MB converts  $\text{Fe}^{3+}$  in methemoglobin to  $\text{Fe}^{2+}$  so that the oxygen bound to  $\text{Fe}^{2+}$  and can be transported) and an anti-respiratory distress agent (McPherson, 2017). The addition of MB to the treatment protocols for severe COVID-19 patients was associated with significant clinical benefits which resulted in decreased duration of hospital stay and mortality (Alamdari et al, 2021).

In continuation of our clinical trials, phase 3 clinical trial was designed to verify the efficacy of MB (the reduced form) for treating hospitalized patients with severe COVID-19 by determining the duration of hospital stay and mortality.

## Methods

### Study Subjects

This study was performed at Mashhad University of Medical Sciences, Mashhad, Iran, after ethics committee approval (IR.MUMS.REC.1399.122; IRCT20191228045924N120, September 20, 2020; ClinicalTrials.gov Identifier: NCT04370288; April 19, 2020) and taking written informed consent from patients. Enrollment for

the clinical trial began on September 20, 2020, and ended on November 20, 2020. The authors were responsible for designing the trial and for collecting and analyzing the data. The clinical trial has been conducted according to the principles expressed in the Declaration of Helsinki.

### Study Design

This study was a randomized, controlled, parallel, open-label trial. The authors could not perform the study blind because of the blue discoloration of the urine in the intervention group. Neither the statistician, nor investigators, nor patients were masked to the treatment assignment. No drugs were masked and a placebo was not used. Inclusion criteria were severe patients with age above 18 years, respiratory distress ( $\geq 26$  breaths/min), oxygen saturation  $\leq 93\%$  at rest in the room air, a confirmed case of COVID-19 (by RT-PCR on the collected nasopharyngeal swab or clinical and typical HRCT features). Exclusion criteria were a history of G6PD deficiency, severe renal failure, cirrhosis, active chronic hepatitis, a history of an allergic reaction to MB, treatment with immunosuppressive agents, pregnancy, breastfeeding, and the presence of any condition that would not allow the protocol to be followed safely such as cognitive impairments or poor mental status. Eligible patients were randomly assigned to either the MB-group (106 patients) or the SC-group (117 patients).

### Methylene blue syrup formulation

The compositions of the syrup were MB, vitamin C, dextrose, N-acetyl cysteine. The special formulation for MB (the reduced form) was patented according to the pathology of COVID-

19 disease (IR-139950140003002083) (on June 1, 2020, PCT).

### Intervention

For the MB-group, along with standard care, MB syrup was administered orally to patients (1 mg/kg every 8 hours for two days, followed by 1 mg/kg every 12 hours for the next 12 days). For the SC-group, the standard care protocol was continued.

The standard care protocols were applied according to WHO guidelines. In the standard care protocols, severe patients receive supplemental oxygen, antiviral agents, intravenous fluids, antibiotics, anticoagulants, corticosteroids (Smith et al, 2020; Janssen et al, 2020). Antivirals: Remdesivir (200 mg on the first day and 100 mg for 4 days), and IFN- $\beta$  (44 $\mu$ g/sc, daily for 3 doses); antibiotics: Azithromycin (500mg/day, 5 days), Meropenem (1 gr, TDS, 7-10 days), Ceftriaxone (1 gr, BID, 7 days), and Vancomycin (1 gr, BID, 7-10 days); and corticosteroids: Dexamethasone (8 mg/day for 10 days), anticoagulants (up to discharge).

The measured outcomes were duration of hospital stay and mortality rate within 28 days. It should be noted that hospital stay was counted from the day following MB treatment.

### Analysis

Continuous variables were compared by the *t*-test and Mann-Whitney test based on data distribution. The paired *t*-test was used to compare the mean difference of these variables (hospital stay and mortality rate) in each study group. The significance level was less than 0.05 in all statistical analyses. SPSS version 23 was used for statistical analysis.

## Results

### Patients

Demographic characterizations of patients in the MB-group and the SC-group are presented in Table 1. Comorbidities in both group were hypertension, cardiovascular disease, diabetes, malignancy, cerebrovascular disease, asthma and chronic obstructive pulmonary disease (COPD).

**Table 1:** Demographic and clinical characteristics of SC-group and MB-group.

Elements	SCG (n=117)	MB G (n=106)	Significant Difference, p value
Age, mean $\pm$ , SD y	63.8 $\pm$ 18.5	61.3 $\pm$ 14.8	No, 0.51
Sex			
Male	62	60	No, 0.34
female	55	46	
No-Comorbidities	43	50	No. 0.13
Comorbidities	74	56	
Outcome Hospital stay (days)	10.6 $\pm$ 9.2	6.2 $\pm$ 3.1	Yes, < 0.001
Recovered	92	93	No, 0.07*
Deceased, %	25, 21.4 %	13, 12.2 %	

Comorbidities: Hypertension, Cardiovascular disease, Diabetes, Malignancy, Cerebrovascular disease, Asthma and COPD. \*: a marginal significant difference

### Outcomes

After MB therapy, the hospital stay (days) was significantly shortened in the MB-group (6.2  $\pm$  3.1) in comparison to the SC-group (10.6  $\pm$  9.2,  $p < 0.001$ ) and a marginal significant decrease of

mortality was seen in the MB-group (12.2%) in comparison to SC-group (21.4%,  $p= 0.07$ ). No serious adverse effects were observed in the MB-group except the color of patients' urine turned to green or blue.

### Discussion

This trial showed that MB, as a supplementary therapy to the standard care protocols, leads to significant decrease of hospital stay and mortality rate.

Severe COVID-19 patients presented with a chief complaint of dyspnea. After 1 day of MB administration, 90 percent of patients expressed dyspnea relief. This finding was very important for the care of COVID-19 patients suffering from respiratory distress.

In our previous trials, we explained in detail, the biochemical processes in the pathogenesis of the disease (Alamdari et al, 2020, Alamdari et al, 2021).

The rationale for considering MB for treatment was due the following mechanisms: 1) Anti-viral activity against the SARS-CoV-2 virus (Bojadzic et al, 2020; Kovács, 1960; Chan et al, 2011), 2) Anti-hypoxemia activity by converting iron from the ferric ( $Fe^{3+}$ ) state to the ferrous ( $Fe^{2+}$ ) state (an approved medicine for methemoglobinemia) (Smith et al, 2020), 3) Anti-respiratory distress activity (Alamdari et al, 2020), 4) Inhibitor of nitrite production (nitrite converts ferrous iron to ferric iron in hemoglobin) by inhibiting nitric oxide synthase and guanylate cyclase in activated macrophages (Mayer et al, 1993), 5) Antimicrobial agent (Woo and Heil, 2017), 6) Inhibitor of reactive oxygen species (superoxide anion and hydrogen peroxide scavenger) (Riedel et al, 2003), 7) Inhibitor of xanthine oxidase (which produces superoxide anion) (Salaris et al, 1991), 8) Anti-platelet aggregation drug (Miculescu and Wiklund, 2010), 9) Antifungal agent

(Haynes et al, 2010), 10) Anti-inflammatory agent (Lin et al, 2017).

### Conclusion

MB therapy along with standard care could be very efficacious in the treatment of COVID-19. Since MB is an inexpensive, ubiquitously accessible, and FDA-approved drug for methemoglobinemia, this drug is an excellent supplementary choice for the treatment of hypoxemia in COVID-19 patients. We suggest that the golden time of MB administration should be upon diagnosis and at least before the severe stage of the disease, where patients present with multi-organ involvement and failure. MB can significantly reduce hospital stay and mortality.

### Limitation of the Study

Limitations were the conducting of the trial in one university center with a small number of patients.

**Trial registration:** ClinicalTrials.gov: NCT04370288; IRCT20191228045924N1

### Role of the Funding source

The funders did not have any role in the design, collection, management, analysis, interpretation of data, writing of the report, or the decision to submit the report for publication.

### Declarations:

**Ethics approval and consent to participate:** Consent informed was obtained from all patients.

Ethics committee approval: IR.MUMS.REC.1399.122

Trial registration:

1- Phase 1: Clinical Trials.gov Identifier: NCT04370288. Registered: April 19, 2020, <https://clinicaltrials.gov/ct2/show/NCT04370288>

2- Phase 2&3: IRCT registration number: IRCT20191228045924N1. Registered: September 20, 2020, <https://en.irct.ir/trial/49767>

**Availability of data and materials:**

Data is available on request through the authors and permission of the ethical committee of the university. If any physician desires to run a randomized clinical trial as part of a multicenter trial, the authors are keen to share their experiences and provide latest updates of their information.

**Competing interests:** All the authors do not have any possible conflicts of interest.

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**Author Contributions Statement**

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