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Research Article

Methylene Blue in Treatment of Severe COVID-19 Patients: A Large Cohort Study

Bhanu Prakash Reddy Attunuru[1*], Madhusudhan Reddy Lingala[1], Podduturi Naveen Chander Reddy[1], Anand V Kulkarni[2], Sasikala Mitnala[3], Duvvur Nageshwar Reddy[2]

[1]*Department of Internal Medicine, AIG Hospitals, Gachibowli, Hyderabad, India*

[2]*Department of Hepatology and Gastroenterology, AIG Hospitals, Gachibowli, Hyderabad, India*

[3]*Asian Healthcare Foundation, AIG Hospitals, Gachibowli, Hyderabad, India*

ABSTRACT

Background and objectives: As there are limited treatment options in treatment severe COVID-19 disease we aim to evaluate the role of methylene blue in management of severe COVID-19 disease. The primary objective was to assess the survival benefit of methylene blue in severe COVID-19 disease. The secondary objective was to compare the duration of hospital stay, the requirement of invasive mechanical ventilation, the development of thrombotic complications and the change in inflammatory markers at discharge/death among the two groups. (SMT group and MB group).

Materials and methods: This is a single-center retrospective observational study of 362 patients admitted with severe COVID-19 disease from July 2020 to February 2021 in a tertiary care hospital during first wave of COVID-19 in India. 254 patients received MB at a dose of 1 mg/kg body weight intravenously for 5 days in addition to Standard Medical Therapy (SMT group) compared with 108 patients who received only SMT, which included remdesivir, low molecular weight heparin and systemic steroids.

Results: The length of hospital stay was shorter in the MB group (12.30 ± 7.106 days) than in the SMT group (14.33 ± 8.683; P=0.021). A similar number of patients developed thrombotic complications in both groups. Fewer patients in MB group required invasive mechanical ventilation (24.8% *vs.* 47.2% in SMT group; P=0.001). There was a significant reduction in the D-dimer (P=0.001) and ferritin levels (P=0.009) with MB. There was a significant mortality benefit with MB on Kaplan-Meier survival analysis. Mortality was 39.2% MB group and 59.3% in the SMT group (P=0.001).

Conclusion: The addition of methylene blue to standard medical therapy has been associated with a reduction in in-hospital mortality, as well as a significant decrease in the length of hospital stay, inflammatory markers and the need for mechanical ventilation in patients with severe COVID-19.

Keywords: COVID-19; Patients; Treatment; Thrombotic complications

INTRODUCTION

The world is affected by the deadly COVID-19 pandemic that is spreading quickly due to SARS-CoV-2. To create evidence-based clinical treatments plans, a deeper comprehension of the pathophysiology of the critical disease state is essential. Severe COVID-19 primarily affects the lungs and vascular endothelium because these cells have a high concentration of ACE2 receptors, which the virus uses to enter the cells. When the body produces excessive reactive oxygen species, reactive nitrogen species,

Correspondence to: Bhanu Prakash Reddy Attunuru, Department of Internal Medicine, AIG Hospitals, Gachibowli, Hyderabad, India; E-mail: drbhanu.prakash@aighospitals.com

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bradykinins and cytokines beyond its ability to neutralize them, these compounds penetrate the interstitial space and cause oxidative stress. This stress damages lung alveoli and the vascular endothelium, leading to necrosis or apoptosis. A probable reason for hypoxia in COVID-19 patients is methemoglobinemia which results from oxidation of iron contained in hemoglobin from the ferrous to ferric form [1]. The oxidation is associated with decrement in capacity of hemoglobin to carry oxygen [2].

The pathophysiology and management of severe COVID-19 have been the focus of extensive research, yet progress remains limited and various therapeutic approaches have shown only partial success. The exact mechanisms driving tissue damage in severe cases are still not fully understood, which makes supportive care measures such as oxygenation, ventilation and hydration essential in patient management. Critically ill patients often face a very low survival rate and require prolonged ICU care, with no universally accepted protocols for their treatment. This situation places a significant burden on health systems, even in the wealthiest and most advanced countries, where extended and frequently unsuccessful ICU care continues to strain resources.

Methylene Blue (MB), a nitric oxide synthase inhibitor, when combined with vitamin C and N-acetyl cysteine increased the survival of severe coronavirus disease (COVID-19) patients. Recently, it has been reported that oral MB can reduce hospital stay and mortality in severe COVID-19 patients [3,4]. Hence, it is hypothesized that MB can effectively reduce the disease progression and improve oxygenation by reducing the adverse effects of bradykinins [5,6]. In addition, MB has antiviral and anti-inflammatory properties [7,8]. Methylene blue a derivative of phenothiazine converts ferric iron in methemoglobin to ferrous iron of normal hemoglobin and is a well-known medication for methemoglobinemia at doses of 1 mg/kg-2 mg/kg in repeatable single-dose infusion shots [9]. Traditionally there are well-known indications for MB like in methemoglobinemia, cyanide poisoning, septic shock [10,11].

To address resource shortages in a hospital setting while managing an increasing patient load, it is crucial to balance the pace of patient intake with resource limitations. This can be achieved by implementing protocol advancements that prioritize patient safety and optimize the use of available resources. Developing clinical therapy methods with a robust rationale and identifying patient subsets at high risk for severe disease necessitates a deeper understanding of the pathophysiology of critical illness. It is crucial to measure the elements of illness pathways that can be modified by various treatments, whether used routinely or in clinical trials. Given the limited treatment options for severe COVID-19 disease our aim was to evaluate the impact of MB in the management of patients with severe COVID-19 [12,13].

MATERIALS AND METHODS

This single-center retrospective observational study included 362 patients with severe COVID-19 disease (severity graded according to the National Institute of Health scoring system) from July 2020 to February 2021 during the first wave in India. These patients are divided into two groups at the discretion of treating Physician. One group received Standard Medical Therapy (SMT group) and other group received Methylene Blue (MB Group). In addition to standard medical therapy patients with known terminal illness, chronic kidney disease, cirrhosis of the liver, malignancy and Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency were excluded. The primary objective was to assess the survival benefit of Methylene blue. The secondary objective was to compare the duration of hospital stay, the requirement of invasive mechanical ventilation, the development of thrombotic complications and the change in inflammatory markers at discharge/death among the two groups [9-11].

Of these total 362 patients, 254 patients received MB (MB group) in addition to Standard Medical Therapy (SMT group), which included remdesivir, low molecular weight heparin and systemic steroids. This was compared with 108 patients who received only SMT. MB was administered at a dose of 1 mg/kg body weight intravenously for 5 days.

Institutional Ethics Committee (IEC) approved the study vide letter number AIG/IEC-Post CT 47/05.2021-03. Baseline demographics and clinical variables of the patients were retrieved. The data was entered into Microsoft Excel. Continuous variables are expressed as mean and Standard Deviation (SD) and categorical variables as percentages (frequency distribution). Student's paired *t*-test and *chi-square* test were used to assess the difference among continuous and categorical variables by using the Statistical Package for Social Sciences (SPSS 23rd version, IBM Corp. USA). Kaplan-Meier analysis was done to compare the survival amongst the two groups [12].

RESULTS

Baseline characteristics including age, sex, comorbidities, oxygen saturation and inflammatory markers are similar in both groups with the mean age in the SMT Group being 61.73 ± 11.708 while that in the MB group is 59.79 ± 10.722. There are 80.6 percent males and 19.4 percent female patients in SMT group and 79.9 percent males and 20.1 percent female patients are MB group. In the SMT group 79.6 percent patients have comorbidities of which 12 percent have HTN, 17.6 percent have diabetes and 25 percent have both hypertension and diabetes. In the MB group 80.3 percent patients have comorbidities of which 16.1 percent have hypertension and 17.3 percent has diabetes and 23 percent have both HTN and diabetes [13].

Mean Spo2 during admission is 86.42 ± 6.047 in the SMT group and 87.43 ± 6.372 in the MB group. Inflammatory markers at admission are similar in both groups with a mean NLR ratio of 12.62 ± 11.033 (n-107), mean CRP is 77.65 ± 85.938 (n-91), mean ferritin is 752.354 ± 639.4 (n-108), mean D dimer is 879.93 ± 1225.5 (n-107) and mean IL6 is 113.09 ± 137.483 (n-104) in SMT group of patients while mean NLR ratio is 11.83 ± 11.25 (n-251), mean CRP is 97.89 ± 87.045 (n-216), mean ferritin is 679.99 ± 502.385 (n-249), mean D dimer is 791.04 ± 1279 (n-247) and mean IL6 is 101.45 ± 194.630 (n-207)

in MB group of patients (Table 1). There was a significant mortality benefit with MB on Kaplan-Meier survival analysis (Figure 1).

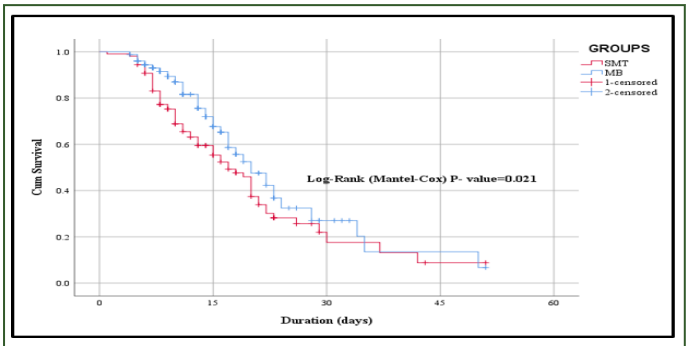

Figure 1: Plots of Kaplan-Meier showing estimates of survival of a group of patients receiving methylene blue and standard medical therapy.

In-hospital mortality was 39.2% MB group and 59.3% in the SMT group (P=0.001). The length of hospital stay was shorter in the MB group (12.30 ± 7.106 days) than in the SMT group (14.33 ± 8.683; P=0.021). A similar number of patients developed thrombotic complications in both groups. (Table 2). Fewer patients in MB group required invasive mechanical ventilation (24.8% *vs.* 47.2% in SMT group; P=0.001). There was a significant reduction in the D-dimer (P=0.001) and ferritin levels (P=0.009) with MB).

Table 1: Clinical characteristics and inflammatory markers.

		AT admission			At discharge		
		SMT group (108)	**MB group (254)**	**P value**	**SMT group (108)**	**MB group (254)**	**P value**
Age		61.73 ± 11.708	59.78 ± 10.722	0.125			
Sex	Male	87 (80.6%)	203 (79.9%)	0.89			
	Female	21 (19.4%)	51 (20.1%)				
Comorbidities	Yes	86 (79.6%)	204 (80.3%)	0.88			
	No	22 (20.4%)	50 (19.7%)				
Hypertension		13 (12.0%)	41 (16.1%)	0.31			
Diabetes		19 (17.6%)	44 (17.3%)	0.95			
Hypertension and diabetes		27 (25%)	56 (23.1%)	0.54			
SPO2		86.42 ± 6.047	87.43 ± 6.372	0.16			
NLR ratio		12.62 ± 11.033 (n-107)	11.83 ± 11.253 (n-251)	0.544	19.86 ± 18.152 (n-102)	15.05 ± 15.563 (n-223)	0.015
CRP		77.65 ± 85.938 (n-91)	97.89 ± 87.045 (n-216)	0.063	33.902 ± 41.176 (n-56)	29.35 ± 107.7 (n-135)	0.76
Ferritin		752.354 ± 639.405 (n-108)	679.995 ± 502.385 (n-249)	0.252	752.354 ± 639.40 (n-87)	679.99 ± 502.38 (n-165)	0.004
D dimer		879.93 ± 1225.526 (n-107)	791.04 ± 1279.543 (n-247)	0.544	1757.85 ± 1961.151 (n-93)	1005.92 ± 1342.283 (n-213)	0.001
IL6		113.09 ± 137.483 (n-104)	101.45 ± 194.630 (n-207)	0.586	183.83 ± 366.131 (n-72)	71.68 ± 293.945 (n-45)	0.08

Note: NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-Reactive Protein; IL: Interleukin; SMT: Standard Medical Therapy, MB: Methylene Blue, SPO2-Oxygen Saturation

Table 2: Outcomes of MB treatment in severe COVID-19.

		SMT group (n-108)	MB group (n-254)	p value
Length of hospital stay		14.33 ± 8.683	12.30 ± 7.106	0.021
Outcomes	Discharge	44 (40.7%)	176 (69.3%)	0.001
	Death	64 (59.3%)	78 (39.2%)	
Thrombotic complications	Yes	11 (10.2%)	24 (9.4%)	0.828
	No	97 (89.8%)	230 (90.6%)	
Invasive mechanical ventilation	Yes	51 (47.2%)	63 (24.8%)	0.001
	No	57 (52.8%)	191 (75.2%)	
Δ NLR		6.990 ± 18.950 (n-102)	3.423 ± 15.224 (n-222)	0.071
Δ CRP		-45.831 ± 88.325 (n-55)	-62.408 ± 123.231 (n-125)	0.369
Δ Ferritin		183.167 ± 712.138 (n-87)	-16.559 ± 482.349 (n-163)	0.009
Δ D-dimer		952.675 ± 1975.697 (n-92)	164.028 ± 1880.289 (n-209)	0.001
Δ IL6		-31.219 ± 381.579 (n-44)	35.900 ± 368.235 (n-114)	0.123

Note: NLR: Neutrophil-to-Lymphocyte Ratio; CRP: C-Reactive Protein; IL: Interleukin; SMT: Standard Medical Therapy; MB: Methylene Blue

DISCUSSION

Our study showed that adding methylene blue to standard medical therapy decreased in-hospital mortality along with a significant reduction in the duration of hospital stay. There was a significant reduction in levels of inflammatory markers like ferritin levels and need for invasive mechanical ventilation in MB group when compared to a SMT group. Furthermore, D-dimer levels and thrombotic complications were lower in the MB group compared to the SMT group, likely due to methylene blue's inhibition of arachidonic acid breakdown by platelets [14-16]. Methylene blue affects platelet function by inhibiting platelet thromboxane A2 and endothelial prostacyclin I2 (PGI-2) synthesis, as well as reducing platelet aggregation and the metabolism of arachidonic acid through the cyclooxygenase and lipoxygenase pathways, which is facilitated by its action on cellular NADPH [14,15].

These results corroborate with the phase 1 clinical trial which demonstrated MB improved survival, contained the inflammatory markers and severity of disease in COVID-19 patients [1]. Furthermore, phase 2 clinical trials with oral MB reduced hospital stay and improved survival in severe COVID-19 patients and very recent Indian study showed improvement in oxygenation, ventilation free days' decrease in mortality and reduction in inflammatory markers [16]. These studies included only five 40 and 50 patients respectively.

The majority of individuals who received methylene blue, often exhibited dark-colored urine and no patient has ever been reported for having encountered an allergic reaction, sense of disorientation, bluish discoloration of the palms, which are common adverse reactions or any other reactions commonly seen with this medication [17].

CONCLUSION

In conclusion, MB is a safe, economical and effective drug with beneficial effects in the treatment of severe COVID-19. Given the ongoing pandemic with a high mortality rate, methylene blue can be used as safe drug in reducing mortality as there are limited options in severe COVID-19 treatment in addition to currently approved medications. Further studies are required to evaluate the prophylactic role of MB in preventing the progression of mild/moderate disease to severe COVID-19. Our study is the first Asian study to demonstrate the beneficial effects of MB in a large cohort of severe COVID-19 patients. If MB is administered early during the disease, outcomes will improve if added before the patient reaches a very severe stage of the disease and has multi-organ involvement and failure. We propose that the best time to start methylene blue is before the worsening of clinical condition. The limitation is that the study was not randomized.

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