

Clinical Outcomes of Parenteral Methylene Blue as Adjunct Therapy in Hospitalized COVID-19 Patients: A Retrospective Cohort Study

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ABSTRACT

Background: During the pandemic, methylene blue emerged as a repurposed drug for the treatment of COVID-19. The objective was to evaluate clinical outcomes of parenteral methylene blue use in hospitalized COVID-19 patients.

Methodology: A retrospective cohort study was conducted at COVID-19 hospitals in Rajkot, during 2022-2023. "Exposed" cohort (who received standard care plus parenteral methylene blue), "Unexposed" cohort (with standard care) were matched for age, gender, and HRCT scores. Primary outcomes included length of hospital stay and recovery rate. Secondary outcomes assessed changes in serial laboratory parameters. Data were analyzed using appropriate statistical tests using Jamovi Version 2.6.45.

Results: A total of 19 exposed and 34 unexposed patients were analyzed. The exposed cohort had significantly longer hospital stays (median 9 vs. 5 days; $p < 0.01$) and higher mortality (21.1% vs. 2.9%; $p = 0.09$). Baseline hemoglobin (11.30 vs 13.80 g/dL at baseline, $p = 0.017$) was significantly lower in the exposed cohort and showed an initial rise followed by a decline, unlike the stable trend in the unexposed group. D-dimer levels (430.00 vs 220.00 ng/mL at baseline, $p = 0.02$) remained significantly higher in exposed cohort. CRP declined similarly whereas IL-6, ferritin, and creatinine showed differing trends.

Conclusions: Longer hospital stays and higher mortality was observed in exposed cohort than unexposed.

Key-words: COVID-19, Methylene Blue, Drug Repurposing, Cohort Study, Length of Stay, Hospital Mortality

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INTRODUCTION

The COVID-19 pandemic was a global health emergency and posed significant challenges to healthcare systems worldwide. In the early stages of the COVID-19 pandemic, in absence of standardized treatment guidelines, various drugs were used off-label in an attempt to improve patient outcomes.[1] Several therapeutic options were explored for severe COVID-19, with controversial results. Repurposing was one of the promising approaches that involved identifying approved drugs. This strategy significantly hastened clinical testing in human pharmacology and toxicology, saving time in the regulatory review process.[2,3] Faced with significant mortality rates and overwhelmed healthcare systems lacking access to clinical trials, many drugs, such as antimalarials (hydroxychloroquine), anthelmintics (ivermectin), antivirals (remdesivir), and immunomodulatory drugs (anakinra and tocilizumab), were utilized off-label during the early stages of the COVID-19 pandemic.[1] Repurposing of well-known, widely available drugs presents an attractive approach, and methylene blue was one of the drugs used for the treatment of severe COVID-19 infection during the pandemic.[2]

Methylene blue was approved for the treatment of acquired methemoglobinemia in pediatric and adult patients.[2,4] Since the 19th century, medical professionals and researchers have utilized methylene blue for a diverse range of clinical purposes. Methylene blue is a dye that alleviates oxidative stress through antioxidant activity, decreases hypoxemia and decreases respiratory distress at safer doses (of <2 mg/kg).[5] It is one of the drugs to be repurposed for different clinical applications several times for the treatment of malaria,[6] cyanide poisoning [7]. Methylene blue has potent broad-spectrum antiviral effects against Zika and dengue virus both in vitro and in vivo.[8] Additionally, methylene blue was used off-label and explored as a potential therapeutic option for severe COVID-19 infections.[2,9] Observational studies with methylene blue have demonstrated beneficial effects on hemodynamic parameters and oxygen delivery in septic shock.[10] The utilization of methylene blue in COVID-19 management was reported in a few cases during the COVID-19 pandemic.[2] There are limited clinical data available regarding the use of methylene blue in COVID-19 patients in India. The present study aimed to evaluate the real-world clinical outcomes of parenteral use of methylene blue in hospitalized COVID-19 patients.

Primary objective of the study was to compare clinical outcomes (mortality and hospital length of stay) between patients receiving parenteral methylene blue as adjuvant therapy versus standard care alone, and the secondary objective was assessment of changes in serial laboratory parameters including hemoglobin, white blood cell count, C-reactive protein (CRP), D-dimer, ferritin, interleukin-6 (IL-6), serum lactate dehydrogenase (LDH), serum creatinine, and random blood sugar (RBS) during hospitalization between both groups.

MATERIALS AND METHODS

Study design: This was a retrospective cohort study conducted during 2022-2023 at designated COVID -19 hospitals of Rajkot, Gujarat. This study is reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement for cohort studies; the completed STROBE checklist is provided as a supplementary file.

Selection criteria for study population:

Inclusion criteria: The indoor case records of the COVID-19 hospitalised adult patients, of either gender having complete data of confirmed diagnosis (by Rapid Antigen Test (RAT) or Reverse Transcription-Polymerase Chain Reaction (RTPCR), or CT thorax- HRCT), treatment (drugs with dose, route, frequency of administration, and duration), investigations (minimum 2 investigation were must -one at the time of admission and any follow up) and final outcomes with discharge summary were included in the study.

Exclusion criteria: Patient with lower respiratory tract infection other than COVID-19 or patients who received treatment other than Standard of Care (SOC) and incomplete records regarding diagnosis, treatment, investigation or outcome, discharge summary, discharged against medical advice (DAMA) cases were excluded from the study. The eligible study population was further categorized into the "Exposed" and "Unexposed" groups as follows:

"Exposed Cohort": COVID-19 hospitalized patients who received standard of care plus parenteral methylene blue (1 ampoule i.e. 50 mg, intravenously for 3 days) during hospitalization. The selection of participants was described in the flow diagram.

"Unexposed Cohort": COVID-19 hospitalized patients who received standard of care alone during hospitalization. (Figure 1).

Sample size calculation was not applicable, as this was a retrospective analysis of all eligible patients who received methylene blue during the study period, rather than a prospectively powered design.

Outcome variable: The exposed and unexposed were compared for clinical outcome (Discharged/death) duration of hospital stay and change in serial investigations during from baseline (on admission to discharge/death). Patients were discharged based on clinical improvement, stable oxygen saturation ($\geq 94\%$) on room air, and no further need for monitoring or parenteral drug administration.

Ethical consideration: The study was conducted after obtaining permission from Institutional Ethics Committee (IEC NO. PDUMCR/IEC/34/2022, dated 30/11/2022) of a tertiary care teaching hospital and permission from in-charge physicians of designated private COVID-19 hospitals. As this was a retrospective study involving review of existing hospital case records with no direct patient

contact or intervention, the Institutional Ethics Committee granted a waiver of informed consent. Patient confidentiality was maintained throughout by anonymizing and de-identifying all data prior to analysis.

Data collection: Hospital records were reviewed, and after evaluating the inclusion criteria, exposed and unexposed patients were matched based on sociodemographic factors (age and gender) and disease characteristics (severity of COVID-19 by HRCT scores at the time of admission). Of the 205 hospitalized COVID-19 patients confirmed by RAT, RT-PCR, or HRCT, 53 patients had complete records of diagnosis, treatment, investigations, and outcome and were eligible for inclusion; the remaining 152 were excluded for incomplete records. The authors manually screened medical records of all COVID-19 patients admitted during the study period who met inclusion criteria. Matching for all three characteristics (age, gender, and HRCT severity) was performed by entering values of these variables in Microsoft Excel and conducting repeated analysis to ensure appropriate matching ratios while minimizing selection bias. As only

34 suitably matched unexposed patients could be identified within the eligible pool of 53 patients, the final analyzed sample achieved an approximate 1:1.8 ratio (19 exposed and 34 unexposed) rather than the targeted 1:2 ratio. (Figure 1)

HRCT severity was calculated using a standardized 25-point scoring system where each of the five lung lobes was scored from 0-5 based on the extent of involvement (0 = no involvement, 1 = <5%, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, 5 = >75% involvement), with total scores categorized as mild (1-12), moderate (13-16), or severe (17-25).[11]

Data regarding sociodemographic (age, gender, comorbidity), disease characteristics, type of laboratory test for confirming the diagnosis of COVID-19 infection, onset and duration of symptoms), clinical data (Saturation of Peripheral Oxygen (SpO₂), laboratory investigations), drug therapy and treatment outcome (discharge/death, length of hospital duration) were collected in predefined case record form.

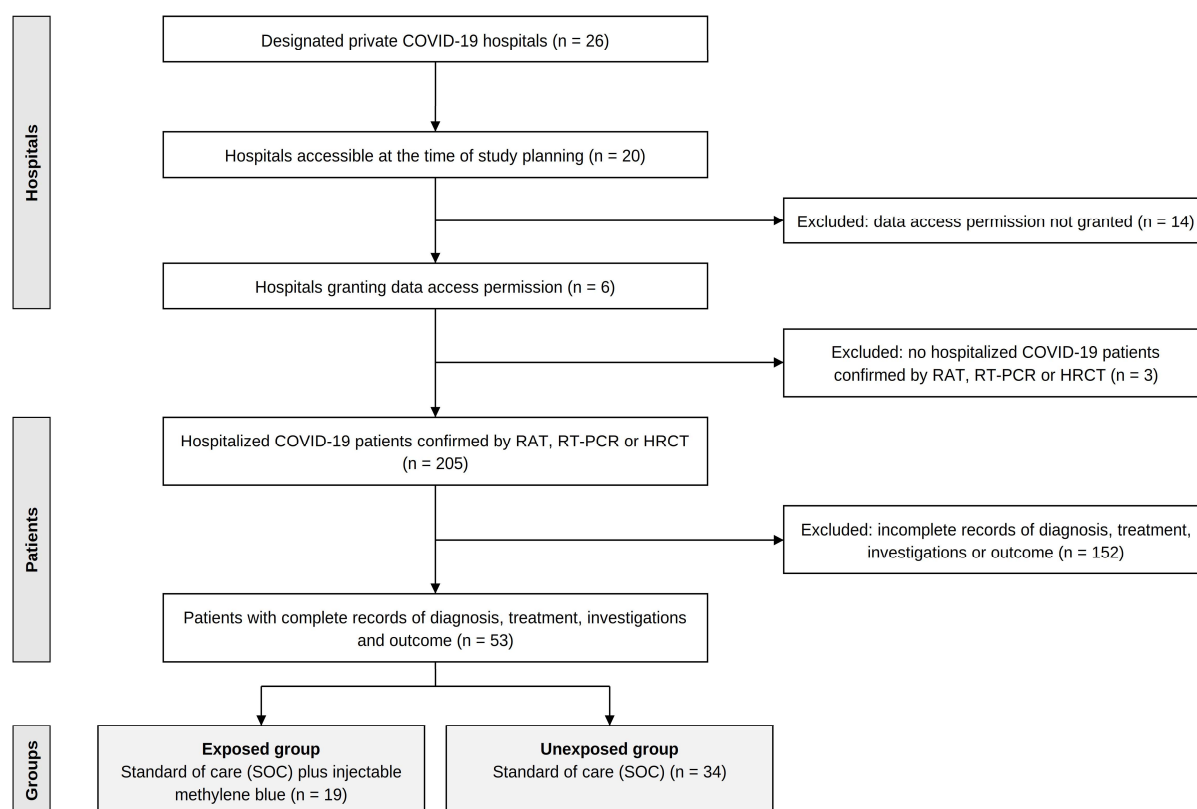


Figure 1: STROBE Flowchart of hospital and patient selection and allocation to the exposed and unexposed groups

HRCT, high-resolution computed tomography; RAT, rapid antigen test; RT-PCR, reverse transcription–polymerase chain reaction; SOC, standard of care.

Statistical analysis: Data were entered in Microsoft Office Excel 2019 and analyzed using jamovi Version 2.6.45. Analysis included all available data at each time point (complete-case analysis); no imputation was performed. Categorical variables were compared using the chi-square test (with Yates' correction where cell counts

were small), and relative risk with 95% CI was calculated for mortality. Continuous and ordinal variables, including serial laboratory investigations and hospital stay, were compared using the Mann-Whitney U test. Multivariable mortality analysis, adjusted for baseline SpO₂, hemoglobin, and D-dimer, used Firth's penalized logistic regres-

sion given the small number of events. Stratified analysis by HRCT severity and baseline SpO₂ used the Mann-Whitney U test (hospital stay, per stratum) and the Cochran-Mantel-Haenszel test (mortality, pooled). A *p*-value <0.05 was considered statistically significant.

RESULTS

A total of 53 patients were included in the final analysis, with 19 patients in the exposed cohort and 34 patients in the unexposed cohort. The baseline sociodemographic (age, gender, comorbidity) and clinical characteristics (type of diagnostic methods, duration of symptoms, HRCT severity score and SPO₂) of both groups were presented in Table 1. Exposed and unexposed were adequately matched for age (mean age: 53.36 ± 13.1 years in exposed vs 52.31 ± 12.37 years in unexposed; *p* = 0.89), gender (78.9% males in exposed vs 76.5% in unexposed; *p* = 1.0), duration of symptoms prior to hospitalization (6.78 ± 3.18 days in exposed vs 6.5 ± 2.68 days in unexposed; *p* = 0.72). Exposed and unexposed showed no statistically significant differences in these variables. Disease severity assessment through HRCT scoring revealed comparable lung involvement between groups, with exposed having a mean score of 13.35 ± 3.24 compared to 13.03 ± 3.12 in unexposed (*p*=0.75). The distribution of HRCT severity categories also showed similar patterns, with the majority of patients in both groups falling into the moderate severity range (64.3% in exposed vs 73.3% in unexposed). However, two significant differences emerged between the groups that warrant careful consideration. First, there was significant difference in diagnostic methods employed (*p*<0.01), with exposed being predominantly diagnosed

through Rapid Antigen Testing (63.2%) and RT-PCR (21.1%), while unexposed were more frequently diagnosed via HRCT imaging (67.6%). More critically, baseline oxygen saturation levels differed significantly between groups (*p*<0.01), with exposed presenting with a median SpO₂ of 92% compared to 98% in unexposed. (Table 1)

Laboratory investigations, including hemoglobin level, white blood cell count, CRP, LDH, RBS, D-dimer, IL-6, and serum creatinine, were performed during hospitalization in both the exposed and unexposed groups. Serial values for hemoglobin, total leukocyte count, D-dimer, and RBS - the parameters showing a statistically significant between-group difference at one or more time points - are presented in Table 2, along with the number of patients (N) contributing to each estimate. (Table 2)

At baseline, the exposed group had significantly lower hemoglobin levels compared to the unexposed group (11.30 vs. 13.80 g/dL, *p*=0.017); hemoglobin initially rose but later declined in the exposed group, whereas levels remained stable in the unexposed group throughout. White blood cell counts were similar between groups at baseline (*p*=0.21), while the exposed group showed consistently lower counts during treatment, with a statistically significant difference at follow-up 1 (6740.00 vs 9560.00/μL, *p*=0.04). D-dimer levels were significantly elevated in the exposed group at baseline (430.00 vs 220.00 ng/mL, *p*=0.02) and follow-up 2 (720.00 vs 320.00 ng/mL, *p*=0.01), remaining numerically higher at later follow-ups. RBS was significantly lower in the exposed group at baseline (132.00 vs 159.00 mg/dL, *p*=0.01).

Table 1: Demographic Profile and Disease Characteristic of Exposed and Unexposed group

Characteristic	Exposed group (n = 19)	Unexposed group (n = 34)	Test statistic	P value
Age, years, mean ± SD	53.36 ± 13.10	52.31 ± 12.37	<i>t</i> = 0.14 ^a	0.89
Duration of symptoms before hospitalization, days, mean ± SD	6.78 ± 3.18	6.50 ± 2.68	<i>t</i> = 0.35 ^a	0.72
HRCT score, mean ± SD	13.35 ± 3.24	13.03 ± 3.12	<i>t</i> = 0.31 ^a	0.75
Gender, n (%)			χ^2 = 0.001 ^b	1.00
Male	15 (78.9)	26 (76.5)		
Female	4 (21.1)	8 (23.5)		
Laboratory confirmation of COVID-19, n (%)			χ^2 = 13.11 ^c	<0.01
Rapid antigen test	12 (63.2)	5 (14.7)		
RT-PCR	4 (21.1)	6 (17.6)		
HRCT	3 (15.8)	23 (67.6)		
HRCT severity, n (%) ^e			χ^2 = 0.06 ^{b,f}	0.80
Mild (1–12)	1 (7.1)	2 (6.7)		
Moderate (13–16)	9 (64.3)	22 (73.3)		
Severe (17–25)	4 (28.6)	6 (20.0)		
Comorbidities, n (%)			χ^2 = 0.50 ^c	0.47
Yes	8 (42.1)	11 (32.4)		
No	11 (57.9)	23 (67.6)		
Baseline SpO ₂ , %, median (IQR)	92 (6.0)	98 (1.5)	<i>Z</i> = 4.78 ^d	<0.01

Data are presented as mean ± SD, n (%), or median (IQR). Percentages may not total 100 because of rounding. Abbreviations: HRCT, high-resolution computed tomography; IQR, interquartile range; RT-PCR, reverse transcription–polymerase chain reaction; SD, standard deviation; SpO₂, peripheral oxygen saturation.; ^a Independent-samples *t* test. ^b Chi-square test with Yates' continuity correction. ^c Pearson's chi-square test. ^d Mann–Whitney U test.; ^e HRCT severity data were available for 14 participants in the exposed group and 30 in the unexposed group; percentages are calculated on these denominators.; ^f Mild and moderate categories were combined and compared with the severe category.

Table 2: Comparison of Serial Investigations between Exposed and Unexposed During Hospitalization

Variable/ Serial Investigation	Exposed group (n=19)			Unexposed group (n=34)			Mann-Whitney U Test	
	N	Median	IQR (Q1-Q3)	N	Median	IQR (Q1-Q3)	Z value	P value
Hemoglobin (g/dL)								
Baseline	13	11.30	3.40 (10.45-13.85)	32	13.80	2.70 (12.23-14.93)	-2.39	0.017
Follow-up 1	11	12.20	3.40 (10.60-14.00)	26	13.85	2.65 (12.18-14.83)	-1.79	0.073
Follow-up 2	7	11.30	3.30 (11.10-14.40)	25	14.00	1.75 (13.00-14.75)	-1.84	0.065
Follow-up 3	3	10.20	*	12	14.00	1.53 (13.45-14.98)	-2.60	0.009
Total Count (Cells/μL)								
Baseline	12	7370.00	3352.5 (5222.5-8575)	31	8740.00	6860.00 (5660.00-12520.00)	-1.24	0.21
Follow-up 1	11	6740.00	3780.00 (5960.00-9740.00)	26	9560.00	5222.50 (7217.50-12440.00)	-1.99	0.04
Follow-up 2	7	7680.00	4920.00 (4930.00-9850.00)	24	9880.00	4692.50 (8405.00-13097.50)	-1.55	0.11
Follow-up 3	3	7410.00	*	12	8550.00	6140.00 (7650.00--)*	-1.73	0.08
S. D-Dimer (ng/mL)								
Baseline	14	430.00	565.50 (237.00-802.50)	34	220.00	360.00 (87.50-447.50)	-2.21	0.02
Follow-up 1	11	400.00	445.78 (290.00-735.78)	28	270.00	307.50 (192.50-500.00)	-1.65	0.09
Follow-up 2	8	720.00	475.00 (402.50-877.50)	31	320.00	417.37 (180.00-597.37)	-2.34	0.01
Follow-up 3	4	600.00	528.75 (397.50-926.25)	13	400.00	260.00 (320.00-580.00)	-1.36	0.17
RBS (mg/dL)								
Baseline	9	132.00	36.00 (107.5-143.5)	31	159.00	112.00 (138.00-250.00)	-2.59	0.01
Follow-up 1	9	124.00	85.50 (98.50-184.00)	21	177.00	93.00 (133.00-226.00)	-1.90	0.05
Follow-up 2	7	142.00	97.00 (104.00-201.00)	18	145.50	42.50 (116.00-158.50)	-0.09	0.92
Follow-up 3	3	122.00	*	10	143.00	71.25 (111.50-182.75)	-1.69	0.86

N = number of patients with a recorded value for that investigation at that time point; this is not necessarily equal to the full cohort size (Exposed n=19, Unexposed n=34) because of missing/unavailable follow-up samples.

* IQR not computable / not reported at this time point due to the small number of contributing observations (see N column).

Table 3a: Comparison of Duration of Hospital Stay and Clinical Outcomes between Exposed and Unexposed Groups

Outcome	Exposed group (n = 19)	Unexposed group (n = 34)	RR (95% CI)	Test statistic	P value
Duration of hospital stay, days, median (IQR)	9 (6.0)	5 (3.0)	-	Z = 3.19 ^a	<0.01
Clinical outcome, n (%)				$\chi^2 = 2.80^b$	0.09
Death	4 (21.1)	1 (2.9)	7.16 (0.86–59.53)		
Discharge	15 (78.9)	33 (97.1)	1.00 (reference)		

Data are presented as median (IQR) or n (%).

Abbreviations: CI, confidence interval; IQR, interquartile range; RR, relative risk.

^a Mann-Whitney U test. ^b Chi-square test with Yates' continuity correction.

Table 3b. Hospital stay and mortality in the exposed and unexposed groups, stratified by HRCT severity and baseline SpO₂

Stratum	Exposed group (n = 19)			Unexposed group (n = 34)			P value (mortality) ^a	P value (LOS) ^b
	n	Deaths, n (%)	Median LOS, days	n	Deaths, n (%)	Median LOS, days		
HRCT severity ^c							0.56	
Mild (1–12)	1	0 (0.0)	5.0	2	1 (50.0)	5.0		1.00
Moderate (13–16)	9	1 (11.1)	9.0	22	0 (0.0)	5.0		<0.001
Severe (17–25)	4	1 (25.0)	11.0	6	0 (0.0)	11.0		1.00
Baseline SpO ₂ ^c							0.67	
<93%	11	3 (27.3)	9.0	1	0 (0.0)	5.0		0.24
≥93%	8	1 (12.5)	8.5	28	1 (3.6)	5.5		0.12

Abbreviations: HRCT, high-resolution computed tomography; LOS, length of hospital stay; SpO₂, peripheral oxygen saturation.

^a Cochran-Mantel-Haenszel test across strata. ^b Mann-Whitney U test within each stratum.

^c Stratum totals reflect participants with available data (HRCT severity: 14 exposed, 30 unexposed; baseline SpO₂: 19 exposed, 29 unexposed).

CRP, LDH, serum creatinine, ferritin, and IL-6 showed no statistically significant between-group difference at any time point. CRP declined progressively in both groups over the course of hospitalization. LDH trended upward in the exposed group while declining in the unexposed group. Creatinine rose modestly in the exposed group during treatment, most notably at follow-up 2

(1.04 vs 0.70 mg/dL, p=0.09), while remaining relatively stable in the unexposed group. Ferritin rose persistently in the exposed group from baseline to follow-up 2 before declining at follow-up 3, while remaining relatively stable in the unexposed group throughout. IL-6 rose from baseline to follow-up 1 before declining in the exposed group, while rising from follow-up 1 to follow-up

2 in the unexposed group; estimates in the exposed group at follow-up 1-3 were each based on a single available observation (N=1) and should be interpreted with caution. Trends for CRP, D-dimer, ferritin, and IL-6 are also presented graphically. (Figure 2)

The analysis of hospital stays and clinical outcomes revealed a statistically significant difference in duration of hospitalization but not in mortality. Patients receiving methylene blue in addition to SOC had a significantly longer median hospital stay than those receiving SOC alone (9 vs. 5 days, $p<0.01$), while mortality was numerically higher but not statistically significant (21.1% vs. 2.9%, $p=0.09$). (Table 3a). On stratifying by HRCT severity category and baseline SpO₂, the longer hospital stays in the exposed group persisted within the Moderate

HRCT severity stratum ($p<0.001$), while pooled mortality differences remained non-significant for both stratification variables (HRCT severity: $p=0.56$; SpO₂: $p=0.67$), with SpO₂ stratification limited by near-complete separation between groups. (Table 3b)

The relative risk for death was 7.16, indicating that patients in the methylene blue group had a 7.16-fold higher risk of mortality compared to the standard care group. However, the wide 95% confidence interval (0.86-59.53) crosses 1.0, indicating that this difference, while clinically concerning, is not statistically significant. The findings suggest that patients receiving methylene blue had both prolonged hospitalization and a trend toward higher mortality compared to those receiving SOC.

Table 4: Multivariable Logistic Regression Analysis for Mortality (Death vs Discharge) - Firth's Penalized (Bias-Reduced) Logistic Regression

Variable	Coefficient (β)	Std. Error	z	df	p-value	Adjusted OR (95% CI)
Baseline SpO ₂ (%)	-0.161	0.229	-0.704	1	0.482	0.851 (0.543-1.334)
Baseline Hemoglobin (g/dL)	-0.463	0.393	-1.181	1	0.238	0.629 (0.292-1.358)
Baseline D-dimer (ng/mL)	0.001	0.001	1.188	1	0.235	1.001 (0.999-1.002)
Treatment Group						
Exposed vs unexposed	-0.442	1.793	-0.246	1	0.805	0.643 (0.019-21.598)

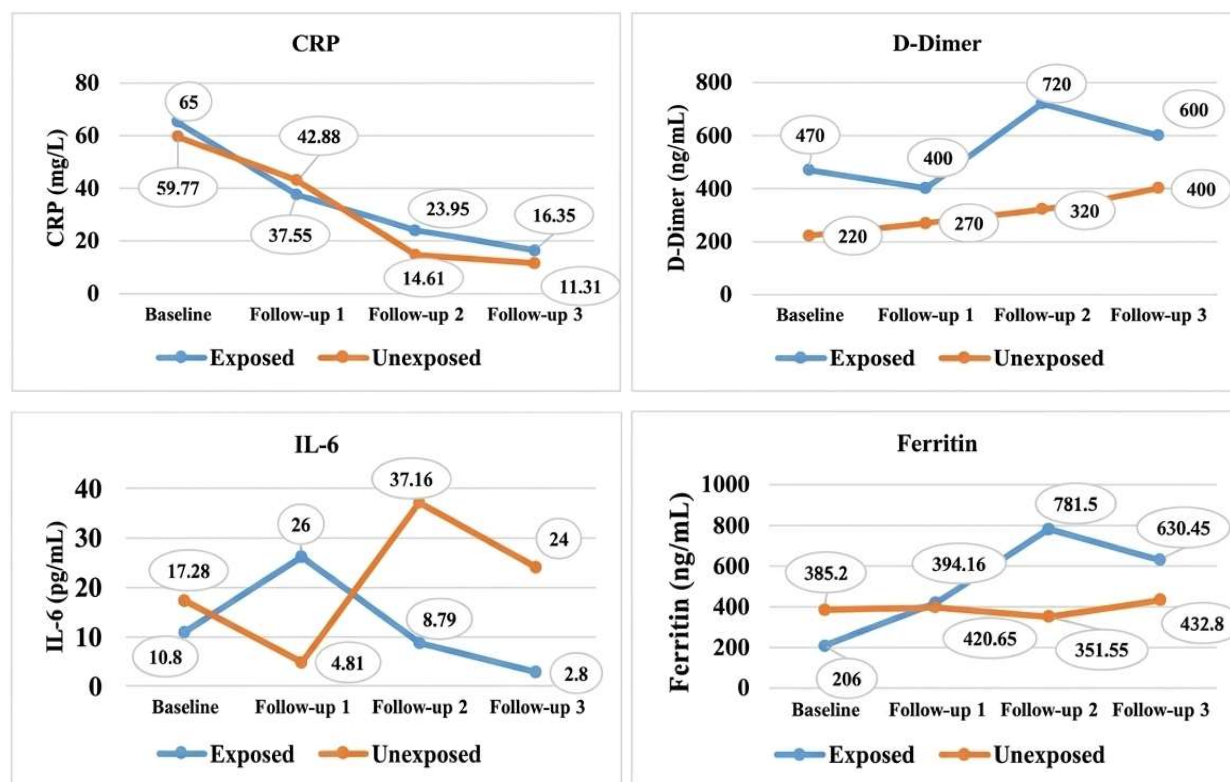


Figure 2: Longitudinal Changes in Biomarkers Among Exposed and Unexposed Cohort

A multivariable logistic regression analysis was conducted to adjust for significant baseline differences between the exposed group and unexposed group, with mortality as the primary outcome. The model included baseline oxygen saturation, hemoglobin levels, and D-dimer concentrations as covariates. Given the small number of mortality events, Firth's penalized (bias-reduced) logistic regression was used to obtain more stable effect esti-

mates. After adjustment, the methylene blue group demonstrated a Firth-adjusted odds ratio of 0.643 (95% CI: 0.019-21.598, $p=0.805$) for mortality in exposed group as compared to unexposed group. As with the unadjusted analysis, the wide confidence interval-driven by the very small number of mortality events-means this estimate should be interpreted as exploratory and hypothesis-generating rather than definitive. (Table 4)

DISCUSSION

This retrospective cohort study showed that the exposed group had a longer hospital stay and a higher mortality rate compared to the unexposed group. The HRCT score was used for matching the severity of COVID-19 infection between “exposed” and “unexposed” as HRCT severity scores correlates with oxygen requirements, morbidity, and mortality in COVID-19 patients.[12] Although HRCT scores were similar between the exposed and unexposed groups, baseline oxygen saturation was lower in the exposed group (average SpO_2 of 92%, vs 98%) suggesting they were more severely ill at the time of admission. This underlying difference may explain the higher mortality observed in the exposed group. The significantly lower oxygen saturation in the exposed group indicates more severe hypoxemia at admission, which is a well-established predictor of poor outcomes in COVID-19 patients.[13] Windradi C et al.[14] demonstrated that baseline $\text{SpO}_2 < 93\%$ was associated with acute respiratory distress syndrome (ARDS), sepsis, and septic shock leading to mortality and prolonged hospitalization in COVID-19 patients, suggesting that exposed group in the study had more severe disease despite comparable HRCT scores. The other possible explanation was chances of potential selection bias, where sicker patients receive experimental treatments, has been a recurring theme in COVID-19 therapeutic studies.

Various theories have been postulated highlighting the repurposing of methylene blue as a salvage therapy among COVID-19 patients for its antiviral, anti-inflammatory, and antioxidant properties and have been proposed as a rescue therapy for improving the refractory hypoxia in COVID-19 patients.[9] Scigliano and Scigliano proposed that methylene blue's ability to reduce oxidative stress and improve cellular oxygen utilization could benefit COVID-19 patients experiencing cytokine storm and hypoxemia.[15] As per the systematic review by Kwok and Howes, the administration of methylene blue was associated with improved hemodynamic parameters in patients with septic shock, by its inhibition of nitric oxide synthase and enhancement of vascular tone.[16] Similarly, Memis D et al. found that methylene blue administration improved oxygen delivery and reduced vasopressor requirements in septic patients.[17] However, these studies involved different patient populations and pathophysiology compared to COVID-19-induced respiratory failure.

The persistently lower hemoglobin levels in the exposed group may reflect underlying anemia or potential hemolytic effects. All study participants receiving methylene blue were tested for Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD) prior to its administration. Gini-muge and Jyothi noted that high-dose methylene blue can induce oxidative stress paradoxically, potentially leading to hemolysis in susceptible patients.[5] In our study patients who received methylene blue had high D-dimer levels as compared to unexposed group. Elevated D-dimer indicates hypercoagulable state and has been

identified as a poor prognostic indicator in COVID-19 patients, as reported by Tang N et al.[18] Garbin JRT et al. established that prolonged hospitalization in COVID-19 patients is typically associated with more severe disease progression and higher complication rates.[19] This finding, combined with the higher mortality trend, suggests that the methylene blue may have been a marker of disease severity rather than intervention effect.

The dosing regimens and routes of methylene blue administration in COVID-19 patients have varied across studies potentially contributing to differences in clinical outcome. In a case series conducted by Mahale N. et al., used intravenous methylene blue (1mg/kg upto five doses) along with SOC as a rescue therapy in 50 hypoxic COVID-19 patients. The study reported significant improvements in $\text{SpO}_2/\text{FiO}_2$ ratios, reductions in proinflammatory markers, and an increase in ventilator-free days. A recovery rate of 60% was observed, with no major adverse effects, highlighting methylene blue's potential as a safe, cost-effective adjunct in the management of severe COVID-19.[9] Barda B et al.[20] conducted a randomized, placebo-controlled Trial (RCT) evaluating the efficacy and safety of oral methylene blue (200 mg in the morning and 100 mg in the evening on day 1, followed by 200 mg daily) in patients with early-phase SARS-CoV-2. Although methylene blue was found to be safe and well-tolerated, the study did not demonstrate superior efficacy or viral clearance compared to placebo. A randomized clinical study conducted by Sanei ZS et al.[21] on 122 hospitalized patients with COVID-19-related acute respiratory distress syndrome (ARDS) reported that oral administration of methylene blue showed beneficial effects in improving SpO_2 levels and reducing inflammatory markers. Although the intervention did not significantly impact survival rates or the duration of hospitalization, the study highlights the potential of methylene blue as a supportive therapeutic option in the management of COVID-19-associated ARDS. A study conducted by Ghodke BA et al.[22] reported the inhalation route of methylene blue administration (nebulization (0.5 mL 0.5% solution of MB + 2.5 mL of distilled water) among 103 hospitalized patients with moderate to severe COVID-19. The study reported significant improvements in oxygen saturation and reductions in inflammatory markers such as serum ferritin, CRP, LDH, and D-dimer, along with an 86.4% recovery rate and a shortened hospital stay, suggesting that methylene blue may be a beneficial adjunct therapy in moderate to severe COVID-19 exposed. The optimal dosing and duration for systemic methylene blue in COVID-19 remains undefined, which may contribute to the inconsistent results observed across different studies.

The inflammatory markers particularly CRP trends, showed similar pattern of reduction between groups despite different clinical outcomes in our study.

The serum levels of IL-6 and CRP have a significant correlation with the severity of COVID-19 and can be used as independent factors to predict disease risk.[23] The exposed cohort had persistently elevated D-dimer levels

throughout the course of treatment, despite receiving systemic anticoagulant therapy. Klok FA et al. [24] reported that COVID-19 is associated with increased thrombotic complications and mortality among patients, and strongly advocated for the use of higher prophylactic anticoagulation doses, even in the absence of randomized trial evidence.

The discrepancy between theoretical benefits and clinical outcomes reflects the complexity of COVID-19 pathophysiology and the challenges of drug repurposing. Successful drug repurposing requires careful consideration of dosing, timing, and patient selection, factors that may not have been optimized in early pandemic treatment protocols.[25]

STRENGTH AND LIMITATIONS

This study provides real-world evidence on the use of parenteral methylene blue in hospitalized COVID-19 patients and included matching of patients based on age, gender, and HRCT severity scores. Additionally, serial laboratory parameters and multivariable logistic regression were used to better assess clinical outcomes and adjust for baseline differences.

The retrospective nature of the study, single-center design, small sample size, and baseline SpO₂ imbalance despite HRCT severity matching may have introduced confounding and limited generalizability. As no a priori sample size calculation was performed, the study was not powered to detect the observed mortality difference between groups; the correspondingly wide confidence intervals reported throughout (e.g., RR 95% CI 0.86-59.53) reflect this limited precision and should be considered when interpreting the reported effect sizes. Additionally, the multivariable mortality model was based on only 3-5 events across 4 predictors, well below the recommended events-per-variable threshold; despite using Firth's penalized regression, the resulting estimates remain exploratory with wide confidence intervals.

CONCLUSION

Patients treated with methylene blue had significantly longer hospital stays and a numerically higher, though not statistically significant, mortality rate compared to those receiving standard of care alone. Notably, HRCT severity - the primary measure used to match disease severity between groups - was comparable, and only baseline oxygen saturation differed significantly, being lower in the exposed group. This pattern is consistent with confounding by indication, whereby more hypoxic and acutely unwell patients may have been preferentially selected to receive methylene blue, rather than the drug itself worsening outcomes; this is supported by the adjusted analysis, in which the mortality association reversed direction (adjusted OR 0.643) after accounting for baseline severity, though the wide confidence inter-

val precludes definitive conclusions. Given the retrospective design, confounding by indication should be regarded as the primary limitation to causal interpretation, and these findings are hypothesis-generating rather than definitive, warranting confirmation through adequately powered randomized controlled trials.

FUTURE RESEARCH DIRECTIONS

Future research with RCTs using standardized protocols, including dose escalation, appropriate patient selection, and adequate sample sizes, is needed to provide definitive evidence for the use of methylene blue in COVID-19.

List of Abbreviations:

ARDS	Acute Respiratory Distress Syndrome
DAMA	Discharged Against Medical Advice
G6PD	Glucose-6-phosphate dehydrogenase
IQR	interquartile range
IL-6	Interleukin- 6
CRP	C- Reactive Protein
HRCT	High-Resolution Computed Tomography
LDH	Lactate Dehydrogenase
RAT	Rapid Antigen Test:
RCT	Randomized Controlled Trial
RBS	Random Blood Sugar
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SOC	Standard of Care
SpO ₂	Saturation of Peripheral Oxygen

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