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Clinical Effects and Outcome of Methylene Blue in Treatment of Covid 19 Patients

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Abstract:
BACKGROUND
COVID-19 is caused by SARS-CoV-2[1] ,a novel beta coronavirus that is the most current of the seven coronaviruses (CoVs) known to infect humans. SARS-CoV-2 is the most recent of the seven coronaviruses (CoVs) known to infect humans. [3]Almost a year after the COVID-19 epidemic began in Wuhan, China, no specific therapy has been discovered, despite millions of deaths globally. Because of the several processes implicated in COVID-19 pathogenic manifestations, including as severe hypoxia, excessive inflammatory response, and defective immune response, a new treatment paradigm is focusing on finding medicines that can perform multiple functions. The antiquated drug methylene blue (MB) appears to fit the above criteria. Methylene blue (MB) also has a potent antifibrotic effect and is very rapid acting. The clinical outcomes of patients with hypoxic coronavirus disease 2019 (covid-19) treated with intravenous methylene blue (MB)[10] in a tertiary care hospital are described in this study.
MATERIALS AND METHODS
A total of 30 patients with severe acute respiratory distress were taken up for the study who were turned out to be RTPCR positive . Data related to objectives of study was collected. Methylene blue was given in intravenous and nebulised form in our hospital from June 08th to September 10th 2020.
RESULTS
1. Methylene blue was found to assist improve patient saturation in our study
2. Reducing the need for higher modes of ventilation
3. Chest X-ray findings were resolved
4. There was also a decrease in in hospital mortality.
5. Future covid related complications such as fibrosis were avoided.
CONCLUSION
We concluded that administering Methylene blue to Covid 19 patients improved oxygen saturation, reduced in hospital mortality, reduced the requirement for higher modes of ventilation, and reduced post-covid sequelae such as fibrosis.
Keywords:*AcetylCholinesterase2,Antiviral,COVID-19,MethyleneBlue,SARS-CoV-2, AcuteRespiratoryDistressSyndrome,CoronavirusDisease2019, RescueTherapy*

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I. Introduction

Coronavirusdisease2019(COVID-19)causedbythesevereacuterespiratorysyndrome-coronavirus(SARS-CoV-2)hasseverelyimpactedhealthcaresystemsalloverttheworld. [12]Additionally, because the lungs are the first organs to be encountered by the virus, they are affected early. The autopsies of many COVID-19 patients reveal inflammation of the endothelium. This is because ACE2 receptors are present on the vascular endothelium lining all the organs[11]While four CoVs (HCoV 229E, OC43, NL63, and HKU1) are responsible for about one third of the common cold cases in humans, three have caused recent epidemics associated with considerable mortality: SARSCoV-1 (2002–2003, causing ~10% mortality), MERS-CoV (Middle East respiratory syndrome coronavirus; 2012, causing ~35% mortality), and now SARS-CoV-2 (2019–2020), which seems to be less lethal but more transmissibleThoughtheexactpathogenesisofSARS-CoV-

2isunknown,varioushypotheseshaveproposedcytokinestormorhyperinflammatorysyndromeasprobablecausesfort herapidworseningofthedisease. CoVs use their glycosylated spike (S) protein to bind to their cognate cell surface receptors and initiate membrane fusion and virus entry. For both SARS-CoV and SARS-CoV2, the S protein mediates entry into cells by binding to angiotensin converting enzyme 2 (ACE2) via its receptorbinding domain (RBD) followed by proteolytic activation by human proteases
Variousdrugshavebeenrepurposedfortreatmentintheabsenceofdefinitivetherapywithemphasisontheprovisionofsup portivecareincludingoxygenation,ventilatorsupport,andothercriticalcarelifesupports.
Methyleneblue(MB)[9]orBis(dimethylamino)phenazathioniumchloridetrihydrate,anorganicdye,hasbeenusedexte nsivelyinanarrayofclinicalconditionsforthepasttwocenturies.FewclinicalconditionswheretheroleofMBhasbeendo cumentedincludetreatmentofmalaria,refractorysepticshock,catecholaminerefractoryvasoplegia,methemoglobine mia,andtherapeuticbenefitinhypoxiacausedduetopulmonaryvasodilationinpatientsofhepatopulmonarysyndromed uetoinhibitionofguanylatecyclaseandnitricoxidesynthetase. [14]MB has a long history of more than 140 years, but it has managed to revive itself because of its wide range of applications. It is one of the most famous drugs to be repurposed for different clinical applications several times. MB was prepared as a dye for textiles by Caro in 1876

Further, Paul Ehrlich developed the staining activity of MB and formed the groundwork of modern chemotherapy in 1891 .Then, in the late 19th century it was used in the treatment of malaria, however, its use was discontinued because of the inevitable adverse effects; blue sclera and green urine . In the 1920s, MB was used as an antidote for cyanide poisoning because the reduction potential of MB is equal to the reduction potential of oxygen, and MB is readily reduced by the elements of the electron transport chain .Further, MB was found to reverse toxic methemoglobinemia . MB has been found to reverse hypotension in sepsis and is useful in cases of vasoplegia .Due to its unique physicochemical characteristics including its ionic charges, redox chemistry, light spectrum properties, and planar structure, MB exerts a wide range of clinical applications on the nervous system .MB has been used in photodynamic therapy for excisional wounds, hepatitis C, HIV, and psoriasis .MB has also been established as a diagnostic marker for oral cancer and breast cancer .Thus, MB is a versatile molecule. Recently, there have been various reports on the use of MB for COVID-19 management. This is because MB inhibits superoxide anion (ROS precursor) formation by blocking the xanthine oxidase pathway, nitric oxide (RNS precursor) formation by directly inhibiting nitric oxide synthase, and cytokine production by attenuating NF-κB pathway .and ultimately inhibits the production of both free radicals and cytokines. This is evident from the various ongoing clinical studies reported in this regard. In this review, the applicability of methylene blue in COVID-19 and its mechanistic aspects have been explored and compiled. The clinical studies have been explained in great detail. Thus, the potential of methylene blue in the management of COVID-19 has been examined
VariousTheorieshavebeenpostulatedhighlightingthebenefitsofadministeringMBasasalvagetherapyamongCOVID-19patientsforitsantiviral,anti-
inflammatory,andantioxidantpropertiesandhavebeenproposedasarescuetherapyforimprovingtherefractoryhypoxi ainCOVID-19patients.
Tilldate,noclinicaltrialshavebeenconductedevaluatingtheclinicaleffectsofMBamongCOVID-19patients
[1]MBinhibitstheviralattachmentandentryofSARS-CoV-2byblockingtheprotein–
proteininteraction(PPI)ofitsspikeproteinwithACE2onthehostcellwhichisthefirstcriticalstepinitiatingtheviralentry.
TheysuggestedthatthisantiviralactivitycouldbeusefulinthepreventionandtreatmentofCOVID-19eitherasanoralorinhaledmedication. inhibit the interaction between SARS-CoV-2 spike protein and its cognate receptor ACE2, which is the first critical step initiating the viral attachment and entry of this CoV. As part of this, we found that methylene blue, a tricyclic phenothiazine compound approved for the treatment inhibits this interaction, and we have confirmed that it does so in a concentration-dependent manner. This can contribute to the antiviral activity of this inexpensive and widely available dyebased drug against SARS-CoV-2 making it potentially useful in the prevention and treatment of COVID-19, especially in nonindustrialized nations
Methyleneblue(mb)[6]photochemicaltechnologyhasbeenproventoinactivatelipid-
envelopedviruseswithhighefficiencyandsafety.thepresentstudyaimedtoinvestigatethesars-cov-2inactivationeffectsofmethyleneblueinplasma Possibly relevant to this, MeBlu was found to improve hypoxemia and hyperdynamic circulation in patients with liver cirrhosis and severe hepatopulmonary syndromeMeBlu is being used for the treatment of pneumonia and other respiratory ailments in less developed countries with some success

II. Materials and Methods

A total of 30 patients with severe acute respiratory distress who were turned out to be RTPCR positive for covid 19 were taken up for the study, data was collected between June 08 to September 10, 2020, and the clinical outcomes were monitored every two weeks till October 30, 2020. related to objectives of study was collected. Methylene blue was given in intravenous and nebulised form in our hospital from june 08 to september 10 2020. Intravenous MB was administered as rescue therapy in dosage of 1 mg/kg body weight, with a maximum of five doses, to patients with high oxygen requirements (spo_2/fio_2 <200) apart from the standard of care after obtaining G6PD levels. Data were abstracted from multiple electronic data sources or patient charts to provide information on patient characteristics, clinical and laboratory variables and outcomes. Standard of care, such as antivirals, steroids, and anticoagulants, was administered apart from oxygen supplementation by nasal cannula, nonrebreather mask (nrbm), high flow nasal cannula (hfnc), or noninvasive ventilation (niv) as per the hospital protocol and discretion of the treating physician. Invasive mechanical ventilation was initiated based on the clinical assessment and spo2/ fio2 of the patients. [2]Demographics, clinical, and laboratory data on admission and the subsequent trends, mode of respiratory support (invasive mechanical ventilation, noninvasive mechanical ventilation, and oxygen mask), fraction of inspired oxygen (FiO2), SpO2/FiO2 ratio and treatment administered were collected from the electronic medical records. The collected data were analyzed and interpreted by two independent intensivists. The clinical team provided clarification on missing or redundant data.

Dose was calculated as 0.08 microgram per ml which was multiplied to ecf volume of around 50 liters which in a 70 kgs individual which comes around 5 ml .This 5 ml was given in intravenous form diluted in 100 ml of normal saline over 15 minutes for a total of 5 days and in nebulised form we gave to the patients by adding it to the ro water .

The study was conducted by the Department of emergency Medicine in a tertiary care hospital located in SSIMS Davangere India.3.1.

Criteria
Inclusioncriteriaare:
1) signedinformedconsent
2) age≥18years
3) microbiologicallyconfirmedSARS-CoV-2infection
4) negativepregnancytestinwomenofchild-bearingage

3.2. ExclusionCriteria

1) documented refusal to participate in the study
2) knownG-6-Phophatasedeficiency
3) treatmentwithaserotoninergicdrug
4) Pregnancyandbreastfeeding
5) HistoryofG6PDdeficiency
6) Severerenalinsufficiency
7) derangedliverfunctiontestorChronicliverdisease
8) Ischemic heart disease patients
9) Chronic pulmonary disease patients

III. Results

Interpretation

Ourstudyshowedthatmethylenebluehelpedinreducingpatientneedforhighermodesofventilationandalsopreventedin hospitalmortalityandcomplicationsrelatedtoCOVIDinfuturelikefibrosis.

Table 1.*Comparision between different age groups*

Age(inyears)	Groups		Total	pvalue
	MethyleneBlue	WithoutMethyleneBlue		
30-50	2	3	5	0.717
	13.3%	20.0%	16.7%	
50-70	7	8	15	
	46.7%	53.3%	50.0%	
Above70	6	4	10	
	40.0%	26.7%	33.3%	

Total	15 100.0%	15 100.0%	30 100.0%

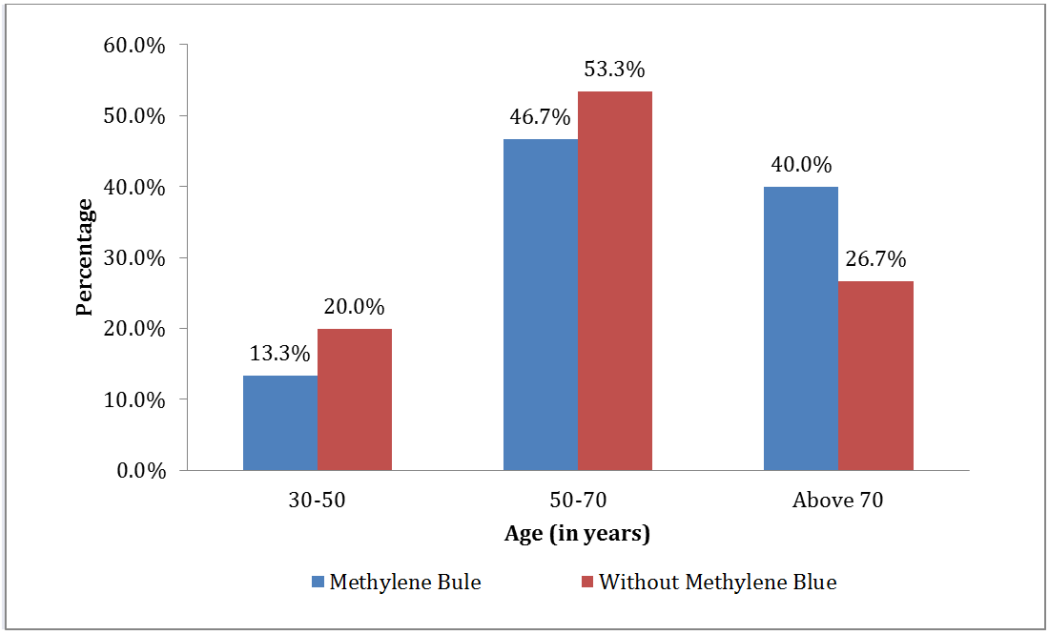

Figure 1. Bar graph showing *Comparision between different age groups*

Table 2. *Morbidity.*

Parameters	MethyleneBlue		WithoutMethyleneBlue		tvalue	pvalue
	Mean	SD	Mean	SD		
Dayofillness	6.33	2.23	8.27	3.49	-1.81	0.083
HRCTScore	11.60	6.50	9.93	6.32	0.71	0.482
NoofVentilatorDays	1.13	1.55	5.93	6.77	-2.68	0.017
TotalNoofHospitalDays	4.73	1.44	14.33	9.79	-3.76	0.002

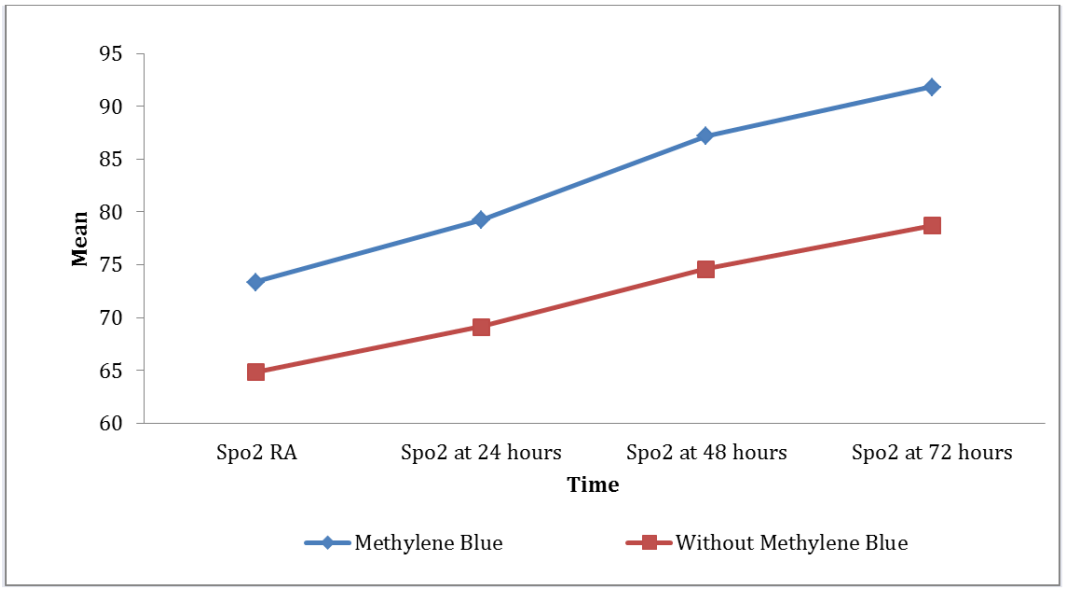

Figure 2.*Graph showing improving trend of SPO2 levels after administration of Methylene blue*

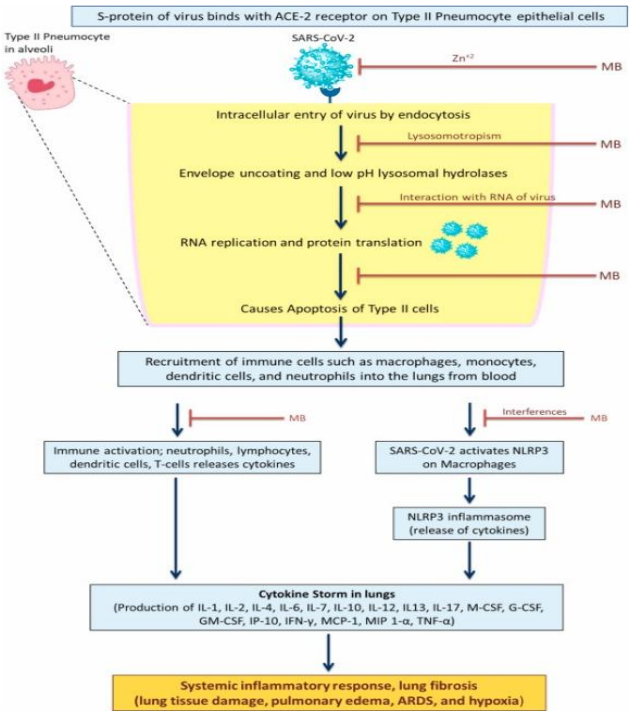

Fig Methylene blue as potential repurposed drug candidate for COVID-19. The diagram depicts the potential interactions of MB with SARS-CoV-2. By protonation, MB accumulates in the lysosome, raising its pH and blocking low pH dependent hydrolases that are required for the virus's uncoating and membrane fusion. MB inhibits the binding of the viral spike (S) protein (glycoprotein) with angiotensin converting enzyme 2 (ACE2) receptors initiates SARS-CoV infection of host cells then being proteolytically activated by human proteases. MB has the ability to transport Zn2+ across the viral envelope by endo-lysosomes. As the Zn+2 metal inhibits the elongation of RNA dependent RNA polymerase. The methylene interferes with the NLRP3 on macrophages and prevents the cytokine storm in lungs.

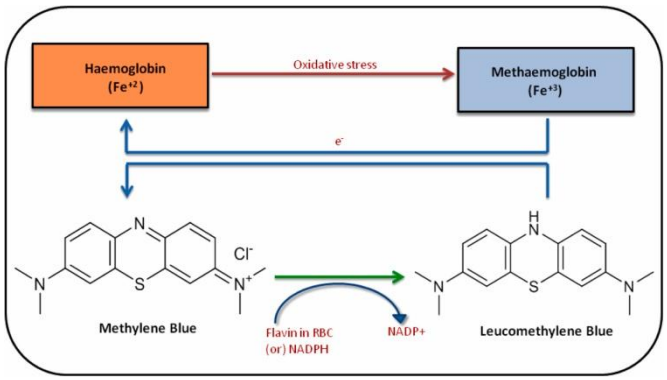

Fig Schematic representation of the Methylene blue accompanied by supplemental oxygen. The methylene blue undergoes reduction by nicotinamide adenine dinucleotide phosphate (NADPH) to produce leucomethylene blue (leucoMB). Note: Fe+2 : Ferrous; Fe+3 : Ferric; RBC: Red blood cells; NADPH: Nicotinamide adenine dinucleotide phosphate; NADP+: Nicotinamide adenine dinucleotide.

IV. Discussion

To our knowledge, this case series is the first in India to publish data on hypoxic COVID-19 patients treated[2] with intravenous mb. The rapid increase in the number of COVID-infected patients, as well as the high caseload in hospitals, has prompted medical researchers to seek out novel medications that could aid in the treatment of COVID-infected patients.

Various medications with well-established safety profiles, widespread availability, and efficacy in treating problems have been investigated. Usb, an organic dye that has been used in a variety of medicinal diseases for over two centuries, has been proposed as a rescue therapy for COVID-19 patients with refractory

hypoxia.methylene blue or methylthioninium chloride is a salt used as dye medication .its a belongs to thiazine group

Antiviral, anti-inflammatory, and antioxidant effects have all been proposed as reasons for using mb as a salvage therapy in COVID-19 patients. mb has broad-spectrum virucidal activity in the presence of light[1] and has been used to inactivate viruses in blood products prior to transfusions.

Mb, according to bojadzic et al., inhibits sars-cov-2 viral attachment and entrance by preventing the ppi of its spike protein with ace2 on the host cell, which is the first essential step in viral entry. By protonation, MB accumulates in the lysosome, raising its pH and blocking low pH dependent hydrolases that are required for the virus's uncoating and membrane fusion.

They hypothesised that this antiviral activity could be used to prevent and treat COVID-19 as an oral or inhaled medicine. Nonphoto-activated mb showed strong in[4] vitro antiviral effective activity against sarscov-2, with inhibitory concentrations (ic) ic50 (0.3 m) and ic90 (0.75 m) that were compatible with oral absorption and iv injection, according to gendrot et al.21. This in vitro activity was higher than previously thought.

Barber et al. and Vardhana et al. found that suppressing the downstream cosignaling ppis of cytotoxic t cells restores their cytotoxicity, activation, proliferation, and cytokine secreting activities, which increases viral clearance.

SHP2 PPI, which is downstream of the PD-1–PD-L1 co-signaling PPI, has low micromolar efficacy but is effective enough to reverse PD-1's suppressive effect on cytotoxic T cells and restore their cytotoxicity, activation, proliferation, and cytokine-secreting activities (Fan et al., 2020). This mechanism of action targeting this co-signaling pathway (PD-1) could contribute to restoring T cell[5] homeostasis and function from an exhausted state (Barber et al., 2006; Vardhana and Wolchok, 2020), which is of interest to improve viral clearance and rein-in the inflammatory immune response and the associated cytokine storm during anti-viral responses like those that cause the high mortality of COVID-19 patients.

Methylene blue was also tried in tuberculosis and various other disease and found beneficial .

V. Conclusions

Ourstudyprovedthatuseofmethyleneblueinpatientshelpedinreducingtheiroxygenrequirement,lengthofhospitalstay wasreduced,improvementinchestxraywasseenmbduetoitspolypharmacologicalactionagainstsars-cov-2,aninexpensiveandwidelyavailabledrugwithminimalsideeffects,hasasignificantpotentialinthetreatmentofCOVID-19.[11]

Furthermetaanalysisneededtoknowfurtheruseofmethyleneblue inCOVIDandrefractoryhypoxicpatients

Futheronfollowupofpatientsthecomplicationsrelatedto COVIDalsowereverylessornotseen.

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