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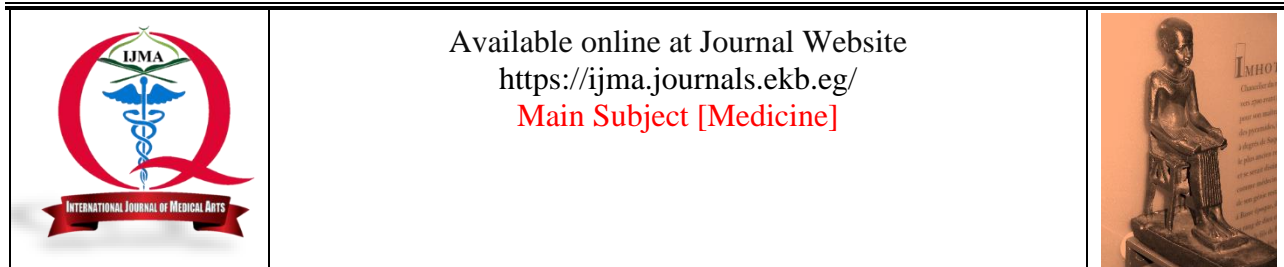
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Original Article

The Role of Nebulized Methylene Blue [NMB] in The Management of COVID-19 Cases: An Observational Study

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ABSTRACT

Background: Many new drugs were studied for desired action in COVID-19 disease dynamics. One such molecule is Methylene blue which is a tricyclic phenothiazine compound approved for the treatment of acquired methemoglobinemia. This molecule has shown some evidence of inhibiting the interaction of the COVID-19 virus and target cells in a dose dependent manner. There are many other proposals suggesting that the mechanisms of action of Nebulized Methylene Blue [NBM] molecule could be beneficial to the management of COVID-19.

The Aim of the Work: To observe and evaluate the effect of NMB on the clinical course and outcomes of patients with COVID-19 infections.

Patients and Methods: An observational study including a total of 63 COVID-19 RT-PCR positive cases divided in 3 groups.

Results: Difference between mean d-dimer value and mean lactate dehydrogenase [LDH] values at day sixth of the intervention among three different groups were statically significant. A general trend of reduced hospital stays in groups getting NBE in groups 1, 2 vs. group 3 was observed [Viz. 9.17 days Vs. 12 days] though statistically non-significant. Fall in inflammatory markers and oxygen requirements in group receiving methylene blue [Groups 1 and 2] vs. control [Group 3] are noticeable.

Conclusion: A general trend of fall in inflammatory markers and oxygen requirements among patients in groups receiving NMB. However, our results showed no significant differences in outcome measures for oxygen saturation and duration of hospitalisation among those receiving NMB.

Keywords: Methylene Blue; COVID 19; Inflammatory Markers; Nebulization.

INTRODUCTION

Severe Acute Respiratory Syndrome CoronaVirus 2 [SARS CoV2] also known as coronavirus disease-19 [COVID19] pandemic remained a persistent challenged worldwide [1].

As a tertiary care teaching medical college in central India, we are managing COVID-19 cases, most of which are referral cases.

Many new drugs were studied for desired action in COVID-19 disease dynamics. One such molecule is methylene blue which is, a tricyclic phenothiazine compound approved for the treatment of acquired methemoglobinemia [2]. Methylene blue [MB] or methylthionine chloride, is chemically [3, 7-bis [dimethyl amino] phenothiazine-5-ium chloride], is a deep blue in colour. [3] This molecule was found to inhibit the interaction of COVID-19 virus and target cells in dose dependent manner [4].

There are many mechanisms of action of this molecule that was proposed to be beneficial in COVID-19 disease process. Methylene Blue in intravenous form was being used for COVID-19 patients [5, 6]. Efficiency of nebulization as a method of drug delivery for methylene blue has been analyzed previously by Moghissi K et al. [7] and they found that methylene blue gets delivered in satisfactory level in bronchial tree by this method. [7]

THE AIM OF THE WORK

With this background, we had planned this study to evaluate the effect of NMB on the clinical course and outcomes of patients with COVID-19 infections.

PATIENTS AND METHODS

An observational study was conducted to evaluate the effects of NMB on outcomes in COVID-19 patients. Among admitted patients in wards of CR Gardi Hospital, RD Gardi Medical College, Ujjain, Madhya Pradesh, 63 RT-PCR confirmed COVID-19 cases of moderate disease severity as defined by the World Health Organization [WHO] were chosen for the study and were divided into equal three groups [each 21 cases]. Study ran from May 2021 to June 2021 in our institute. All the groups received standard care and treatment as per COVID-19 protocol.

The first group included patients, who had NMB 0.5 mg along with bronchodilator Levosalbutamol [1.25mg] plus ipratropium [500 mcg] three times a day, in their treatment plan. Group 2 includes patients who had NMB 0.5 mg along with inhalational steroid budesonide [1mg] in their treatment plan. Group 3 consists of those patients who had no NMB as control was planned in their treatment plan.

All the groups were age and comorbidity matched. All patients in 3 groups were selected from COVID-19 wards of our institute to ensure similarity in clinical condition.

Eligibility and inclusion criteria: Patients ages 18 to 90 years old, of both sexes, with cconfirmed case of Covid-19 [by RT-PCR, RAT], admission to HDU, ICU with oxygen requirements, and oxygen saturation [SpO2] <94% on room air

Exclusion Criteria were:

1. Pregnancy and breastfeeding women were excluded as Methylene blue exposure, since MB is a Class X teratogen and its exposure in the second trimester is known to be associated with increased risk of fetal malformations, still birth and neonatal complications.

2. History of glucose-6 phosphate dehydrogenase deficiency [G6PDH] deficiency, were excluded as Methylene blue is considered contraindicated for patients with enzyme G6PD deficiency and it is known to cause haemolysis in these patients.

3. Severe renal insufficiency [glomerular filtration rate <30 mL/min/1.73m^2], as its excretion is via renal route. Renal failure is relative contraindication for methylene blue administration.

5. Active chronic hepatitis, chronic liver disease, and those having GOT or GPT levels, five times the upper limits were excluded, as precautionary measure.

6. Patients with history of allergic reaction or significant sensitivity to methylene blue.

Outcome measured as death versus discharge, disease severity indication measured as intensive care units [ICU] requirements, SpO2 levels measured on day 0, day 3, and day 6 of intervention, levels of inflammatory markers and day of stay in hospital. SpO$_2$ levels as measured from SpO$_2$ sensors from Mindray MEC-1200 Patient Monitor. SpO$_2$ levels are accepted as quick and reliable estimate of patient's oxygenation status. SpO$_2$ levels in blood are depended on ratio between oxygenated and deoxygenated haemoglobin levels and hence reflect both oxygen and carbon dioxide levels in the blood indirectly. In this study we used SpO$_2$ levels as point of care prognostic markers for the COVID-19 patients.

Oxygen Flow rates - As all patients were having oxygen saturation <94% on enrolment, hence all were provided with oxygen support. Oxygen flow rates as required for maintenance of SpO$_2$ >94% is regarded as direct marker of disease severity and pneumonia. In this oxygen flow rates were used as a direct marker of inflammatory status and disease progression. Inflammatory markers are raised in all inflammatory state and they are universally raised in COVID-19. In this study CRP and serum lactate dehydrogenase [LDH] levels are taken as inflammatory marker to mark disease progression.

D-Dimer and LDH levels: COVID-19 is a pro-thrombotic state and thrombotic and thromboembolic phenomenon is found to be directly associated with pathogenesis and disease outcomes. Therefore d-dimer

level serial measurements are used to indicate serial thrombotic status of disease. In this study we used serial d-dimer measurements for the same as marker.

Informed consent was obtained from all the patients included in the study. This study was done at the peak of COVID-19 pandemic in India. Institute ethics committee approval was obtained for this study [IECREF NO-1A/2021].

Statistical Methods: This study used SPSS 23 package for analysis. For analysis of differences among 3 groups, One-way analysis of variance [ANOVA] was used to compare means.

RESULTS AND DISCUSSION

Comparison of means of different variables is done alongside three groups & analysed by ANOVA test. The mean age of participants in group 1, 2 and 3 are 47.61 years, 52 years and 56 years respectively. Difference between mean d-dimer value and mean LDH values at day 6th of the intervention among three different groups were statically significant. A general trend of reduced hospital stays in groups getting NMB Group 1, 2 vs. Group 3 was observed [9.17 days Vs. 12 days] though statistically non-significant [Table 1]. The fall in inflammatory markers and O_2 requirements in group receiving methylene blue vs. control that is Group 3 is noticeable. [Table 2]

Table [1]: Comparison between study groups regarding patient age, glycated hemoglobin, hospital stay duration, oxygen saturation, mean flow rate, and inflammatory markers

Factors	Group 1	Group2	Group 3	ANOVA Significance	Remark for significance
Mean Age [Years]	47.61	52	56	0.224	
HbA1C	7.87%	9.47%	10.27%	0.431	
Duration of Stay [discharged cases] [day]	9.17	10.32	12	0.232	
Mean Spo2 Day 0	91.06%	93.69%	92.67%	0.480	
Mean Spo2 day 3	90.44%	93.4%	91.18%	0.699	
Mean Spo2 day 6	93%	92.94%	91.18%	0.197	
Mean O_2 flow requirements day 0 [L/min]	11.25	7.13	10.61	0.058	
Mean O_2 flow requirements day 3 [L/min]	8.13	7.13	10.46	0.430	
Mean O_2 flow requirements day 6 [L/min]	4.62	4.24	5.33	0.867	
Mean CRP value day 0 [mg/L]	148.14	131.23	98.25	0.546	
Mean CRP value day 3[mg/L]	70.74	58.5	93.69	0.794	
Mean CRP value day 6 [mg/L]	19.33	40.67	44.98	0.492	
Mean d-dimer value day 0 ng/ml	2818.27	2120.46	3254.08	0.490	
Mean d-dimer value day 3 ng/ml	1605.83	2483.64	2789.26	0.709	
Mean d-dimer value day 6 ng/ml	1963.99	1251.50	5732.20	0.015	Significant
Mean LDH day 0 [U/L]	1257.5	616.38	670.53	0.174	
Mean LDH day 3 [U/L]	360.75	479.92	354.00	0.519	
Mean LDH day 6 [U/L]	225	339.47	409.66	0.023	Significant

Table [2]: Trend of factors on Day 0 vs. Day 6 in different group.

Factors	Group 1	Group 2	Group3
Mean SpO_2 day 0	91.06%	93.69%	92.67%
Mean SpO_2 day 6	93%	92.94%	91.18%
Mean O_2 flow rate day 0	11.25	7.13	10.61
Mean O_2 flow day 6	4.62	4.24	5.33
Mean CRP day0	148.14	131.23	98.25
Mean CRP day 6	19.33	40.67	44.98
Mean LDH day 0	1257.5	616.38	670.53
Mean LDH day 6	225	339.47	409.66
Mean d-dimer day 0	2818.27	2120.46	3254.08
Mean d-dimer day 6	1963.99	1251.50	5732.20

Methylene Blue [MB] has been used in the treatment of various diseases since time immemorial and has been administered mainly through oral and intravenous routes. MB is considered a safe drug when used in doses of < 2 mg/kg [8]. However, it may produce toxic effects at high doses. At 2–4 mg/kg, it can cause skin desquamation, and at 7 mg/kg it may cause nausea, vomiting, fever, abdominal pain, haemolysis, and chest pain. As the dose increases to 7.5 mg/kg, it may cause confusion and hyperpyrexia. At a dose of 20 mg/kg, it may lead to hypotension. At a very high dose of 80 mg/kg, it may cause a bluish skin discoloration like cyanosis [3, 9, 10].

A continuous infusion of MB at 0.25–1 mg/kg/hour is indicated for sepsis treatment [11]. For anaphylactic shock, MB is given as intravenous bolus injection at a dose of 1.5–2 mg/kg [12]. In our institute, we use safe dose of 0.15

mg/24 hour in 3 divided doses, by nebulising MB at concentration of 0.5mg by nebuliser. MB was found to inhibit replication of COVID-19 virus in vitro in dose dependent manner [6]. It was also found to inhibit interaction of virion with host cells, by inhibiting interaction of SARSCoV2 spike protein and ACE inhibitor receptor interactions [4]. As COVID 19 causes inflammatory response to body and activates macrophages in high numbers leading to cytokine storm that damages the host cells. Oxidative stress and nitric oxide [NO] contribute to this cycle. It is well reported that NO is majorly involved in viral-induced pneumonia [13]. Nitric oxide synthesis is activated by the cytokine's interferon-α [IFN-α], interleukin-1 [IL-1], IL-2, IL-6, and tumor necrosis factor-α [TNF-α], which are released during COVID-19-associated hyper-inflammation [14]. Nitrite, nitrate [the metabolites of NO] and methaemoglobin, are found to be significantly raised in

COVID19 patients, admitted to ICU [15]. However, the use of NO inhibitors is limited because of their lack of specificity in blocking the various NOs isoforms [L - NMMA, L-NAME]. Thus, NOs inhibitors are not currently in clinical usage, posing a risk of broad tissue necrosis and a greater death rate [16]. Alamdari and colleagues [15] concluded that methylene blue, N-Acetyl cysteine and vitamin-C can act as anti-oxidants and limit the oxidative damage caused at cellular level. Methylene blue is known as the only drug known to target mediators of inflammation and reactive oxygen species and hence inhibits cytokines in a wide manner [17].

MB undergoes renal excretion as a mixture of MB, leucoMB and demethylated MB metabolites like azure A and azure B [2]. After oral administration, the maximum concentration of MB is reached at 2 hours. Its plasma half-life is 20 hours [18]. 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.

Conclusion: A general trend of fall in inflammatory markers and oxygen requirements in patients receiving methylene blue. However, results showed no significant differences in outcome measures for oxygen saturation and duration of hospitalisation among those receiving NMB. Despite having a smaller sample size, the current study has suggested that NMB could be imperative in the management of COVID 19. A study with a relatively larger sample size is recommended to build up a comprehensive picture of the effect of NMB on COVID-19 patients.

Conflicts of Interest: None.

REFERENCES

1. Da Costa VG, Moreli ML, Saivish MV. The emergence of SARS, MERS and novel SARS-2 coronaviruses in the 21st century. Arch Virol. 2020 Jul;165[7]:1517-1526. doi: 10.1007/s00705-020-04628-0.

2. Schirmer RH, Adler H, Pickhardt M, Mandelkow E. Lest we forget you--methylene blue. Neurobiol Aging. 2011; 32 [12]: 2325.e7-16. doi: 10.1016/j. neurobiolaging.2010. 12.012.

3. Miclescu A, Wiklund L. Methylene blue, an old drug with new indications. J Rom Anest Terap Int. 2010; 17: 35-41.

4. Bojadzic D, Alcazar O, Buchwald P. Methylene Blue Inhibits the SARS-CoV-2 Spike-ACE2 Protein-Protein Inter-action-a Mechanism that can Contribute to its Antiviral Activity Against COVID-19. Front Pharmacol. 2021; 11:600372. doi: 10.3389/ fphar. 2020.600372.

5. Dabholkar N, Gorantla S, Dubey SK, Alexander A, Taliyan R, Singhvi G. Repurposing methylene blue in the management of COVID-19: Mechanistic aspects and clinical investigations. Biomed Pharmacother. 2021; 142: 112023. doi: 10.1016/ j.biopha.2021.112023.

6. Gendrot M, Andreani J, Duflot I, Boxberger M, Le Bideau M, Mosnier J. Methylene blue inhibits replication of SARS-CoV-2 in vitro. Int J Antimicrob Agents. 2020; 56:106202. doi: 10.1016/j. ijantimicag.2020. 106202.

7. Moghissi K, Dixon K, Gibbins S. Does PDT have potential in the treatment of COVID 19 patients? Photodiagnosis Photodyn Ther. 2020 Sep;31:101889.

8. Ginimuge PR, Jyothi SD. Methylene blue: revisited. J Anaesthesiol Clin Pharmacol. 2010 Oct;26[4]:517-20.

9. Mathew S, Linhartova L, Raghuraman G. Hyperpyrexia and prolonged postoperative disorientation following methylene blue infusion during parathyroidectomy. Anaesthesia. 2006 Jun;61 [6]: 580-3. doi: 10.1111/j. 1365-2044.2006.04619.x.

10. Maslow AD, Stearns G, Butala P, Schwartz CS, Gough J, Singh AK. The hemodynamic effects of methylene blue when administered at the onset of cardio-pulmonary bypass. Anesth Analg. 2006 Jul; 103 [1]: 2-8. doi: 10.1213/01.ane.0000221261.25310.fe.

11. Kwok ES, Howes D. Use of methylene blue in sepsis: a systematic review. J Intensive Care Med. 2006 Nov-Dec;21[6]:359-63. doi: 10.1177/0885066606290671.

12. Oliveira Neto AM, Duarte NM, Vicente WV, Viaro F, Evora PR. Methylene blue: an effective treatment for contrast medium-induced anaphylaxis. Med Sci Monit. 2003 Nov;9[11]:CS102-6.

13. Fang W, Jiang J, Su L, Shu T, Liu H, Lai S, et al. The role of NO in COVID-19 and potential therapeutic strategies. Free Radic Biol Med. 2021 Feb 1; 163:153-162. doi: 10.1016/j.freeradbiomed. 2020.12.008.

14. Vaz AR, Silva SL, Barateiro A, Fernandes A, Falcão AS, et al. Pro-inflammatory cytokines intensify the activation of NO/NOS, JNK1/2 and caspase cascades in immature neurons exposed to elevated levels of unconjugated bilirubin. Exp Neurol. 2011; 229 [2]:381-90. doi: 10.1016/j.expneurol. 2011. 03.004.

15. Alamdari DH, Moghaddam AB, Amini S, Keramati MR, Zarmehri AM, Alamdari AH, et al. Application of methylene blue -vitamin C -N-acetyl cysteine for treatment of critically ill COVID-19 patients, report of a phase-I clinical trial. Eur J Pharmacol. 2020 Oct 15; 885:173494. doi: 10.1016/j.ejphar.2020.173494.

16. Evora PR. Methylene Blue Is a Guanylate Cyclase Inhibitor That Does Not Interfere with Nitric Oxide Synthesis. Tex Heart Inst J. 2016 Feb 1;43[1]:103. doi: 10.14503/THIJ-15-5629.

17. Scigliano G, Scigliano GA. Methylene blue in covid-19. Med Hypotheses. 2021 Jan; 146:110455. doi: 10.1016/ j.mehy.2020. 110455.

18. Walter-Sack I, Rengelshausen J, Oberwittler H, Burhenne J, Mueller O, Meissner P, et al. High absolute bioavailability of methylene blue given as an aqueous oral formulation. Eur J Clin Pharmacol. 2009; 65 [2]:179-89. doi: 10.1007/ s00228-008-0563-x.

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