Role of Methylene Blue Nebulisation in Post COVID-19 Patients for Improving Lung Function and Oxygenation; A Pilot Study

Original Article

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ABSTRACT

Introduction: In covid-19 pneumonia, due to excessive formation of cytokines and hypercoagulability, lung damage occurs and may progress to multiorgan failure and vasoplegic shock. There have been studies where methylene blue, when given intravenously to treat covid patients, showed positive outcomes. Still, no study or case report has shown its effect when given via nebulization.

Materials and Methods: In this pilot study, In all patients, Routine blood investigations, Arterial blood gas (ABG), serum lactate dehydrogenase (LDH) level, coagulation profile, d-dimer and chest x-ray were sent. As a standard of care protocol, the patient received supplemental oxygen support, intravenous fluids, antiviral agent, antibiotics, anticoagulants and steroid therapy if needed. We have used methylene blue nebulisation in Post COVID-19 patients to improve oxygenation. All post covid patients were nebulized with methylene blue [1ml (10mg) + 4ml Normal Saline] 12 hourly and an ABG sample was obtained after 1 hour of nebulization with standard of care ICU management.

Results: Four of six patients improved and were discharged from ICU, while two patients expired. Their bad outcome may be due to comparatively worse conditions at admission, having high SOFA scores and APACHE II scores that themselves predict high mortality. HRCT has been improved in all four survived patients and this improvement was found statistically significant ($P< 0.001$).

Conclusion: In our study we concluded that Methylene blue nebulisation seems to be effective novel method for post covid patients in reducing fibrosis and increasing oxygenation with better intensive care outcome.

Key Words: Apache ii, covid-19 ards, covid-19 lung fibrosis, methylene blue, sofa score.

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INTRODUCTION

Primary route of entry of SARS-CoV-2 is the respiratory tract, however it can also enters through faecal route[1]. Acute respiratory distress syndrome (ARDS), a kind of respiratory failure that strikes critically ill patients quickly, can sometimes develop from covid-19 infection[2,3]. Septic shock and multiple organ damage are linked to ARDS[4]. First structure to be affected by the virus is lung after entry through the respiratory tract because of high expression of ACE2 receptors on alveolar epithelial cells.

Free Radicals and cytokines are primarily involved in endothelial damage, being common basis of multiorgan failure in COVID-19. After initiation of Acute Respiratory Distress Syndrome (ARDS), reactive oxygen species (ROS), reactive nitrogen species (RNS) and cytokine production is increased to an extent that to alleviate all with a single cytokine inhibitor is expected to fail[5]. Methylene blue is the only medication that has the ability to prevent the synthesis of all three of these chemical types. By inhibiting the xanthine oxidase pathway, methylene blue reduces the production of superoxide anion, a precursor to reactive oxygen species[6]. Additionally, it suppresses the production of nitric oxide (RNS precursor) by directly inhibiting NO-synthase[7] and attenuates NF-kB signaling[8,9] to prevent the expression of cytokines. The FDA (Food and Drug Administration) and EMA (European Medicines Agency) have approved the tricyclic phenothiazine methylene blue for the treatment of malaria and hemoglobinemia. Methylene blue is also used in the presence of UV light to inactivate viruses in blood products intended for transfusion. Rationale to use MB in covid-19 is motivated by the fact that recent in-vitro studies suggest its effect against virus in absence of UV light[10,11].

MB also has a potent antifibrotic effect. When used sublingually and nebulized, MB is highly effective in clearing the alveolar-capillary block[12].

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There have been studies where methylene blue has been given intravenously to treat covid patients and has been shown to improve outcomes. Still, there has been no study to show its effect when given via nebulization. In this study, we have used methylene blue nebulisation in post covid patients to improve oxygenation.

Primary objective of this study is to evaluate the effect of MB nebulisation in post covid patients on clinical outcome in form of discharge of patients from ICU. Secondary outcome is to assess the the effect of MB nebulisation on P/F ratio and HRCT score on post covid patients.

MATERIALS AND METHODS

After taking ethical approval from our Institutional ethical committee, this study has been done at our tertiary care centre in India. This prospective, interventional, pilot study was performed on 6 confirmed cases of COVID-19 by real time reverse transcription-polymerase chain reaction (RT-PCR) and High resolution computer tomography (HRCT) but patients were RT-PCR negative at the time of admission. Patients were admitted to intensive care unit (ICU) with saturation on room air less than 94%, respiratory rate (RR) more than 20/minute, and exertional dyspnoea. Patients with a history of Glucose 6 phosphate dehydrogenase (G6PD) deficiency, severe renal failure, cirrhosis, active chronic hepatitis, immunosuppressive medication treatment, patients with a history of MB allergy, and female patients who were pregnant or nursing were excluded from the study.

In all patients, routine blood investigations, arterial blood gas (ABG), serum lactate dehydrogenase (LDH) level, coagulation profile, d-dimer and chest x-ray were sent. HRCT was also done on admission to find out the changes in lung after covid 19. As a standard of care protocol, the patient received supplemental oxygen support, intravenous fluids, antiviral agent, antibiotics, anticoagulants and steroid therapy if needed. All patients were nebulized with methylene blue [1ml (10mg) + 4ml Normal Saline] 12 hourly,[13]and an ABG sample was obtained after 1 hour of nebulization. We have not found any side effects, allergic reactions or changes in the colour of urine to blue or green in any patient.

RESULT S

We have recruited 6 post covid patients in the study. The details of these cases are as follows

Case 1

We admitted a 55 year male patient to our post covid ICU with respiratory distress on oxygen support by venturi mask @15 lit/min. After being treated for covid pneumonia for 10 days, he was shifted to post covid ICU when become covid negative. On admission SOFA score was 4, APACHE score was 5 (Table 1**)** and in HRCT covid severity score was 12. No previous significant medical history was found. Routine blood investigations, arterial blood gas (ABG), serum LDH level, coagulation profile and d-dimer was sent. Chest x ray was also done. Chest X ray showed bilateral consolidation in mid and lower segment. As standard of care protocol, patient received supplemental oxygen, intravenous fluids, antiviral agent, antibiotics, anticoagulants and steroid therapy if needed. Patient was nebulized with methylene blue [1ml (10mg) + 4ml NS] 12 hourly for 7 days and ABG sample was obtained after 1 hr of nebulization.

Table 1: Demographic and ICU Data at Admission

Cases	Age (Years)	Sex	SOFA Score	APACHE II Score
1	55	M	4	5
2	50	F	6	20
3	55	M	6	18
4	40	M	7	22
5	65	M	10	27
6	61	M	10	28

At the day of admission, SpO_2 was 89% on venturi mask @15 litre/minute, PaO_2 was found 68.2 and P/F ratio- 113.6, nebulization with methylene blue was started and after 1 hr post nebulization PaO2 was found 76 and P/F ratio 126. No adverse reaction, side effect, or blue or green urine color shift has been observed. Following MB therapy, SpO2 increased from 84% to 93% and the P/F ratio from 126 to 185 on the second day. After that patient was put on simple face mask and then on nasal prongs. On 4th day, patient maintained on room air with SpO2 95% and P/F ratio 237. Serum LDH and TLC were decreased to normal and chest X ray was improved significantly. With a P/F ratio of 250, the patient was discharged from the intensive care unit on the fifth day of MB therapy. (Figure 1)

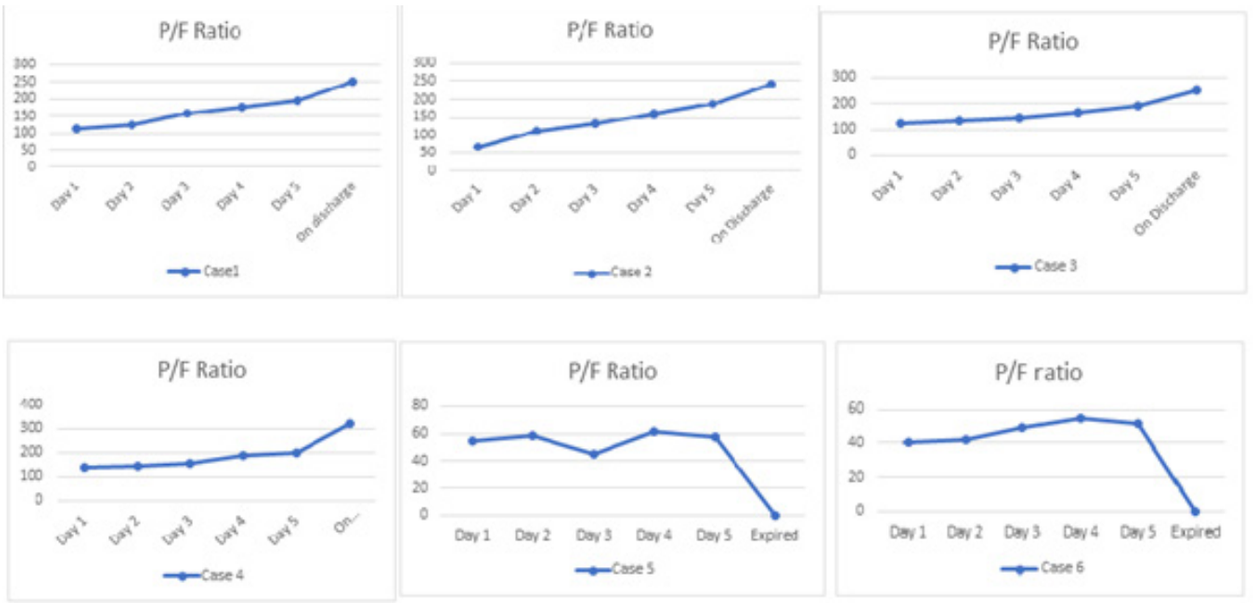

Fig. 1: P/F Ratio of all cases, **Case 1** shows increase of P/F ratio from day 1 to day of discharge, **Case 2**, **Case 3** and **Case 4** shows continuous increase of P/F ratio till day of discharge, **Case 5** shows uneven change of P/F ratio till expired, **Case 6** shows increase of P/F ratio till day 5, then expired

Case 2

50 year old post covid female with respiratory distress having SpO2 84% on Non-invasive ventilation and 0.6 FiO2, SOFA score 6 and APACHE score 20 was admitted in our ICU (Table 1). She had a no past medical history. With a COVID severity score of 17, the lung HRCT showed diffuse bilateral ground-glass opacities and consolidation in the peripheral lung areas. Both upper and lower lobes were involved. After admission we have done standard of care and methylene blue nebulisation according to fixed protocol as in previous case.

At the day of admission PaO2 was 38.7 and P/F ratio was 64.5. She was kept on continuous NIV support with high Fio2 =0.6 because of progressive respiratory distress. On the 4th day of intensive management and MB nebulization, patient was step down to high flow nasal canula oxygen therapy, with intermittent NIV support (Fio2 < 0.5) and P/F ratio of 155.Antibiotic therapy and supportive management were continued. On 7th day of MB nebulization her oxygen requirement on HNFC decreased, and was on intermittent HNFC and reservoir mask. P/F ratio was increased to 185. On 15th day of MB therapy she was discharged on oxygen @2 lit/min with P/F ratio of 240 **(**Figure 1b**)** and SpO2 96%. S. LDH was decreased from 440 to 150.

Case 3

A 55-year-old male post-covid patient was admitted owing to respiratory discomfort and a decreased state of awareness with SpO2 89% on NIV (Fio2 0.6), PaO2 75 and P/F ratio 125. SOFA score and APACHE score was found 6 and 18 respectively (Table 1). HRCT revealed ground-glass opacities in his left lung with covid severity score of 15. Patient was known hypertensive for last 5 years. For this was taking Tab Amlodipine 5 mg once a day. After admission we have done standard of ICU care with supportive treatment and methylene blue nebulisation according to fixed protocol as in case 1. On third day of standard ICU care with MB nebulization, patient became afebrile and oxygen requirement decreased. Patient was now placed on face mask@ 5 litre/minute oxygen support. P/F ratio increased to 205 with SpO2 93%. (Figure 1c). Patient's condition was improving progressively. The patient was taken off oxygen on the ninth day of MB therapy, and on the fifteenth day, was discharged from the intensive care unit.

Case 4

40 year old male known diabetic, admitted to our ICU with respiratory distress with oxygen on reservoir mask @15 lit/min. On arrival to the ICU, SOFA score was 7 and APACHE score was 22 (Table 1). On HRCT covid severity score was found 17 and On the start of study, he was put on NIV with Fio2 0.5, spo2 was 92%, Pao2 was 102 and P/F ratio was 204. We controlled blood sugar with the help of Tab Metformin 500 mg Bd and inj Lantus 16 IU subcutaneous OD. We initiated treatment and investigations according to our ICU standard protocol and MB nebulisation according to our protocol. Patient started improving and P/F ration was increased to 256 on 4th day of treatment with FiO2 40% and SpO2 96%. Furthermore patient was weaned off from NIV and placed on venturi mask @ 8 Litres/minute. Patient further improved and placed on oxygen mask @ 5 litres per minute. After 15

days of intensive care and MB nebulization, patient was discharged on room air with P/F ratio of 305. (Figure 1d).

Case 5

65 year old male admitted to our ICU on non-invasive ventilation with P/F ratio of 55, SOFA score of 10 and APACHE score of 27 (Table 1). We intubated the patient and put him on invasive ventilation with FiO_2 100%. On HRCT, covid severity score was found 20 with ground glass opacities on both lungs. We started treatment according to our ICU protocol and MB nebulisation. Patient deteriorated with high TLC and had reduced P/F ratio. We also added antifungal medication for reduced immunity and chances of fungal infection. We added inj. caspofungin 70 mg (Loading) followed by 50mg 24 hourly. P/F ratio was reduced to 45 and we had to continue the FiO_2 to 100% on 4th day of treatment(Figure 1e). Afterwards patient landed up into septic shock and Inj. Noradrenaline was started on 12 ml per hour by infusion with concentration of 8mg noradrenaline in 50 ml normal saline. Requirement of noradrenaline was increased and patient was not able to maintain vitals on high ventilatory and ionotropic support. After standard of care treatments in ICU with methylene blue nebulisation, patient expired due to cardiopulmonary arrest after 8 days of ICU course.

Case 6

61 year old male admitted on non-invasive ventilation with P/F ratio of 40, SOFA score of 10 and APACHE score of 28 (Table 1). To maintain oxygenation, we intubated patient and put him on mechanical ventilation with FiO_2 100%. Patient was not able to maintain oxygen saturation and vitals. We started noradrenaline infusion to maintain blood pressure. P/F ratio was around 40-60 during the course (Figure 1f)Patient also landed up in acute kidney failure with urine output 500 ml per day and serum creatinine of 2.8. We started Continuous Renal Replacement Therapy (CRRT) as vitals were not stable. Patient deteriorated day by day and finally expired due to cardiopulmonary arrest after 6 days of ICU course and methylene blue nebulisation.

As shown in Table 1, we can see the age , sex, SOFA score and APACHE 2 score of patients at admission in ICU. Four of six patients improved and were discharged from ICU, while two patients expired. Their bad outcome may be due to comparatively worse conditions at admission, having high SOFA scores and APACHE II scores that themselves predict high mortality. Moreover, Case 5 and 6 had very low P/F ratios at admission, 55 and 40, respectively, with high HRCT severity scores of 18 and 19. So chances of improvement in these two patients were already very low so they couldn't be improved. As shown in (Table 2), HRCT has been improved in all four survived patients and this improvement was found statistically significant ($P< 0.001$).

Table 2: HRCT Covid severity score of all cases

Patient	HRCT Covid severity score On Admission	HRCT Covid severity score On Discharge
Case 1	12	8
Case 2	17	10
Case 3	15	10
Case 4	17	11
Case 5	20	Expired
Case 6	19	Expired
Mean± SD	15.25 ±2.36	9.75±1.25
P Value	< 0.001	

HRCT severity was graded as mild (< 8), moderate (10–16), and severe (> 15).

DISCUSSION

In the present study, we have assessed the effect of MB nebulization in post covid patients with the standard of ICU care. In COVID-19 pneumonia, lung damage occurs due to the excessive formation of cytokines and hypercoagulability[14,15]. Antiviral medication, steroids, antibiotics, and vitamin C are used to prevent cytokine storm[16]. For hypercoagulability, low molecular weight heparin (LMWH) was used successfully[17]. But for the fibrosis that happened after covid pneumonia, no agents have been used successfully and effectively till now. We have used MB nebulization to decrease post covid fibrosis in patients. In our case series of 6 patients, four were improved and discharged from ICU with increased P/F ratio, significantly decreased oxygen support, decreased LDH and improved HRCT severity score.

In a study by Ghodke *et al*, they have evaluated the effect of methylene blue nebulisation in moderate to severe cases of covid 19 and found accelerated recovery in patients with decreased levels of inflammatory markers, improved oxygen saturation, reduced length of hospital stay, and clinical improvements [18]. We have got the similar results in post covid patients with use of methylene blue nebulisation.

Up until recently, it was thought that the pathophysiology of COVID-19 was significantly influenced by the "cytokine storm." Nevertheless, there is currently little evidence to support the theory that cytokines or autacoids are the primary cause of the storm[19]. More recently, research has been done on the kininogen system's function in SARS-CoV-2, which shows that ACE-2 receptors are dysregulated resulting in a rise in bradykinin activity and the uninhibition of the kininogen-kallikrein pathway, which is recognized to be a critical factor promoting COVID-19 clinical symptoms such as coagulopathy and dry cough. [20,21].

Bradykinin is a crucial component in the pathophysiology of COVID-19[22]. A phenothiazine derivative, methylene blue neutralises the effect of bradykinin by blocking

the NO synthetase enzyme, which prevents nitric oxide from being synthesized in pulmonary capillaries. This improves oxygenation and acute respiratory distress syndrome[7,23]. Previous nomenclature for bradykinin was endothelial-derived relaxation factors (EDRF)[24]. A number of mediators, including NO, have an impact on the production of cyclic guanosine monophosphate (cGMP), which is the result of the soluble intracellular enzyme guanylate cyclase being activated. MB works by preventing the relaxation of vascular smooth muscle and inhibiting cGMP[25,26]. Leuko-methylene blue is produced when MB converts hemoglobin present in red blood cells. MB acts as a reducing agent on oxidized hemoglobin, returning the ferric ion to its ferrous state, which carries oxygen[27]. Bradykinin activity may be blocked by non-steroid anti-inflammatory drugs (NSAIDs) through different mechanisms. The illness process may persist due to over-activation of these alternate pathways. It has recently been noted that the role that MB plays may be enhanced by the addition of NSAID[19,28]. MB enhances alveolar capillary perfusion, and oxygen-carrying capacity, therefore improves hypoxia[27].

Bradykinin antagonist MB is inexpensive, widely accessible, non-toxic, and simple to use in rural areas. MB should not be administered with SSRIs or other antidepressants, G6PD deficiency and pregnancy.

Along with bradykinin, two other vasoactive peptides substance P and neurotensin are likely to cause microvascular permeability and inflammation and are responsible for ARDS and fibrosis in COVID-19 patients [29]. MB may terminate the effects of bradykinin by inhibiting nitric oxide synthase inhibitor and promoting saturation of oxygen[19].

Since our study is a pilot study and no studies has been done using methylene blue as a nebulisation for post covid patients, we are unable to do comparison with other studies.

Limitation of this study is that we have studied only 6 cases in detail. More data will be required for effectiveness of study. Moreover it is a monocentric study. We need multicentric trails. In severe cases with high APACHE 2 and SOFA score, role of methylene blue couldn't be observed.

CONCLUSIONS

In our study we concluded that Methylene blue nebulisation seems to be effective novel method for post covid patients in reducing fibrosis and increasing oxygenation with better intensive care outcome. The better clinical outcome occurred because of increased oxygenation, reduced inflammatory markers and reduced HRCT covid severity score. So methylene blue nebulisation in post covid patients can be considered as a therapeutic option.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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