Original Research Article

Glutathione an Effective Adjuvant Therapy for Acute Respiratory Distress Syndrome Associated with COVID-19 Infection

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ABSTRACT

Aim: This study is aimed at evaluating the efficacy and safety of Intravenous Glutathione as an add-on to the "Standard of Care" treatment in moderate COVID-19 patients with respiratory distress.

Study Design: A randomized, multicentric, double-blind, placebo-controlled, comparative Phase III clinical trial.

Place and Duration of Study: This clinical trial was conducted at 7 geographically distributed sites across India between February 2021 to September 2021.

Participants: The study enrolled 240 participants who were tested and confirmed cases of moderate COVID-19 with respiratory distress.

Interventions: Intravenous Glutathione (GSH) at a loading dose of 2400 mg on the first day, followed by a dose of 1200 mg every 12 hours for seven days.

Methodology: Moderate COVID-19 patients with respiratory distress were randomized in two groups in a ratio of 1:1, to receive either Glutathione or Placebo. Both the study drugs were given as an add-on to the standard of care (SOC). The SOC was kept as close as possible to the COVID-19 treatment guidelines specified by the Government of India. The study site staff, investigator and patients were blinded to the treatment allocation. The primary endpoint of the study was ≥2-point improvement on the WHO 7-point ordinal scale whereas the secondary endpoints were the proportion of patients achieving scores 3 and below (No Oxygen Requirement) in the WHO 7-point ordinal scale, the proportion of patients shifting from higher to a lower score on WHO 7-Point ordinal scale, the proportion of patients remaining hospitalized, incidences of the need of non-invasive ventilation or new requirement of high flow oxygen use, and incidences of adverse events.

Results: A significant clinical improvement in addition of the GSH treatment to SOC ($p<0.001$) was observed in early treatment days. On day 3, GSH+SOC treatment resulted in 2 or more points of improvement on the WHO 7-point Ordinal Scale in nearly half of the patients as compared to 31.96% on placebo ($p=0.007$; Pearson-chi^2 test; odds ratio, 2.06; 95% CI, 1.22-3.48). Similar results were obtained, in the subset analysis in patients with a baseline score of 5 or more. A higher proportion of patients treated with GSH (64.63%) showed 2 or more point's improvement as compared to the placebo (46.58%) ($p=0.024$; Pearson-chi^2 test; odds ratio, 2.10; 95% CI, 1.10-4.00). The patients who received GSH as an add-on to the SOC were found to be attaining a score of 3 or below 3 (No need of Oxygen supplementation) in higher proportion as compared to those in the placebo + SOC group on Day 2 (odds ratio, 1.26; 95% CI, 0.69-2.29), Day 3 (odds ratio, 1.61; 95% CI, 0.97-2.68) and Day 4 (odds ratio, 1.67; 95% CI, 0.89-3.14). A reduction of severity (based on WHO 7-point ordinal scale) in clinical status was also observed on Day 3 ($p = 0.0269$ by Wilcoxon rank sum test), Day 4 ($p = 0.013$ by Wilcoxon rank sum test) and Day 5 ($p = 0.022$ by Wilcoxon rank sum test). The risk of remaining in the hospital was reduced by 37% with the addition of GSH in conventional therapy for COVID-19. The 7-day dose of GSH was well tolerated by the patients in this study.

Conclusion: GSH supplementation may represent a treatment approach for addressing

cytokine storm syndrome and respiratory distress in patients with COVID-19 pneumonia. Once patients develop clinically confirmed pneumonia or dyspnea, in addition to regular therapy, supplementation should be given intermittently or continuously to enhance tissue availability of GSH. Due to its favorable safety profile, the use of GSH should also be explored in other settings like ARDS due to any other cause in a larger randomized clinical trial.

Keywords: Glutathione, Acute Lung Injury, Acute Respiratory Distress Syndrome, Reactive Oxygen Species, COVID-19

1. INTRODUCTION

Acute lung injury (ALI) is a spectrum of lung diseases characterized by an inflammatory process causing diffuse alveolar damage resulting in hypoxemia and poor lung compliance. [1] ALI is a hallmark of the acute phase and its most severe form, acute respiratory distress syndrome (ARDS) and remains a significant source of morbidity and mortality in the critically ill patient population all over the world. [2] Certain known risk factors such as sepsis, pneumonia, trauma or multiple traumatic injuries may lead to the development of ALI and ARDS. [3]

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Lung injury has been widely recognized as a major clinical problem worldwide. More than 1 million patients are admitted each year with a diagnosis of pulmonary edema. [4] An estimated 1,90,000 patients are diagnosed with lung injuries which are associated with 39% mortality. [5] Approximately 10% of all intensive care unit admissions suffer from acute respiratory failure, with approximately 20% of these patients meeting the criteria for ALI or ARDS. The incidence of ALI in patients with risk factors is 32.7% and that of ARDS is 30% in India and increases in-hospital mortality from 11% (ALI) to 41.8% (ARDS). [6]

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Lungs represent a unique tissue for oxidant stress amongst most organs because they are directly exposed to higher oxygen tensions. [7] The balance between antioxidants and oxidants prevents the disruption of normal physiologic functions. The state of imbalance is collectively referred to as oxidative stress and is associated with lung injuries. [8] Histologically, the hallmark of ALI is the accumulation of neutrophils (polymorphonuclear neutrophils) in the microvasculature of the lung. [9] Inflammation of the lung causes a proliferation of inflammatory mediators that promote neutrophil accumulation in the microcirculation of the lung. These neutrophils activate and migrate in large numbers across the vascular endothelial and alveolar epithelial surfaces [9,10] and release cytotoxic agents such as free radicals, inflammatory mediators, cytokines, granular enzymes, bioactive lipids and proteases due to respiratory burst. [11,12,13] Pro-inflammatory cytokines activate the immune system and participate in the acute inflammatory response, stimulate antigen presentation, upregulation of adhesion molecules, activation of the endothelium, recruitment of inflammatory cells, which significantly contribute to rapid early immunopathogenesis and imbalance of the pro-and anti-inflammatory cytokines which promotes the severity of the disease. [14,15,16]

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A dominant role in the pathogenesis of ALI/ARDS is an oxidative injury to the lung mediated by reactive oxygen (ROS) species resulting in increased capillary leakage, altered surfactant metabolism and diminished pulmonary surfactant function. [11,17] These free radicals upregulate the expression of pro-inflammatory cytokines and adhesion molecules amplifying the tissue damage and pulmonary edema. [13] A proper oxidant-antioxidant balance is critical for vasculature homeostasis making the systems responsible for excessive ROS production can be therapeutic targets in ALI/ARDS treatment.

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Glutathione (GSH) is the most abundant antioxidant and a major detoxification agent in the cells. GSH is required for several cell processes interconnected with alterations in the maintenance and regulation of the thiol-redox status. [18] GSH is synthesized in the cytoplasm by the action of g-glutamylcysteine synthetase and glutathione synthetase, both enzymes requiring ATP. Once synthesized, GSH is distributed in the endoplasmic reticulum, nucleus, and mitochondria. [19] GSH is a tripeptide (cysteine, glycine, and glutamic acid) and the –SH group of its cysteine is extremely sensitive to oxidation, mainly by peroxides. The resulting oxidized form of GSH, Glutathione disulfide (GSSG), characterized by a disulfide bond between two molecules of GSH, efficiently reduced back by the enzyme GSH reductase to GSH. [20,21] As a reducing agent, it is the main cellular antioxidant agent, directly scavenges superoxide anion (O2•-), hydroxyl radicals (•OH), nitric oxide radical (NO•) and detoxifies hydrogen peroxides (H2O2), peroxynitrites (ONOO-), and lipid peroxyl radical (LOO•). [18,19] Oxidative stress is manifested by the excessive production of free radicals and triggers a lethal 'Cytokine Storm' in viral infection. Intracellular redox status alterations are associated with depletion of GSH and contribute to a condition connected with the pathogenesis of respiratory failure. [21] Furthermore, reduced GSH provides an inhibitory effect on angiotensin-converting enzyme (ACE) activity but the oxidized form GSSG shows an activating effect on ACE activity. [22] The patients with ALI/ARDS are deficient in GSH, [13] therefore, the balance between ACE/ACE2 is shifted toward ACE leading to vasoconstriction, oxidative stress, inflammation and apoptosis. By reducing ROS production, GSH activates the ACE2 pathway, inhibits NF-kB activation and consequently keeps the cytokine storm under control.

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Glutathione system (GSH/GSSG) is an important and the most abundant antioxidant in the lungs that decreases in lung inflammatory conditions. [23] Oxidative stress affects the repair mechanisms and the immune control system, which are one of the main events of the inflammatory response which increases the severity of COVID-19. [24] Notably, the disease severity or mortality comes from cytokine storms triggered by viral lung infection, which accounts for multiorgan failure across the body. [25] Despite recent advances in the understanding of the mechanism and treatment of COVID-19-related ARDS, its incidences and mortality rate remains high in the inflammatory phase of COVID-19. [26] Polonikov (2020), studied four moderate-severe COVID-19 cases and found that the three patients with normal/high plasma levels of GSH recovered rapidly, the one with low GSH levels, high plasma ROS and ROS/GSH ratio exacerbated COVID-19 illness. [27] In another case report (2020), two COVID-19 pneumonia patients recovered successfully with the treatment of high doses of supplemental intravenous glutathione. [28] The antioxidant drug, a precursor of GSH, N-acetylcysteine (NAC) has been used for repletion of GSH for years to overcome oxidative stress effects in ALI/ARDS patients. [23,29,30] Thus, one strategy to reduce oxidative lung injury is to restore and maintain the oxidant-antioxidant balance by providing an exogenous source of GSH. Therapeutic benefits of GSH against COVID-19 cytokine storm and its associated risk are outlined in Figure 1.

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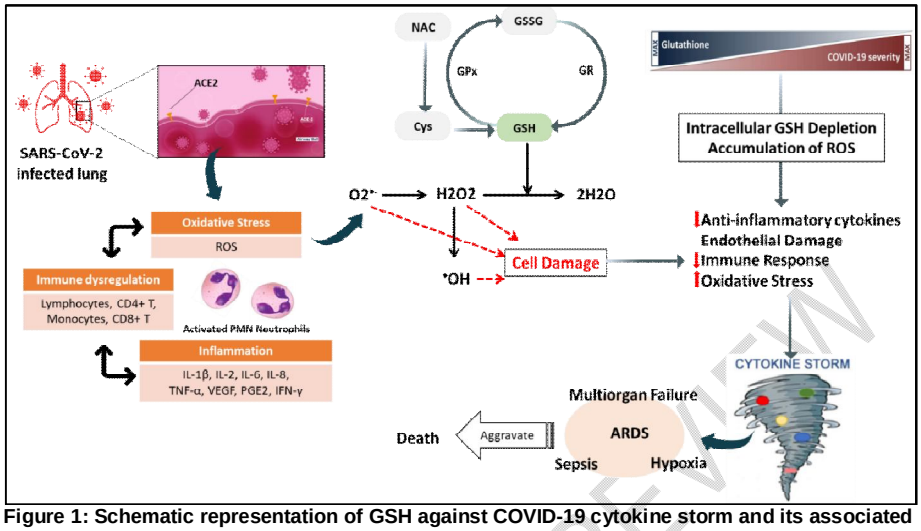

Figure 1: Schematic representation of GSH against COVID-19 cytokine storm and its associated risk. ACE2: Angiotensin-converting enzyme 2, ARDS: Acute respiratory distress syndrome, Cys: Cysteine, GPx: Glutathione peroxidase, GR: Glutathione reductase, GSH: Glutathione, GSSG: Glutathione disulfide, H_2O_2: Hydrogen peroxides, IFN-γ: Interferon-gamma, IL: Interleukin, NAC: N-acetylcysteine, •OH: Hydroxyl radicals, O2•-: Superoxide anion, PGE2: Prostaglandin E2, ROS: Reactive oxygen species, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, TNF-α: Tumors necrosis factor-alpha, VEGF: Vascular endothelial growth factor

In the current COVID-19 pandemic, the patients are burdened with cytokine storm, the best therapeutic strategy for the immune system would be to supplement it with intavenous glutathione. Considering the benefits and need for the therapeutic option for the treatment of ALI/ARDS, this study was conducted in India, to evaluate the safety and efficacy of intravenous formulation containing a predominately reduced form of GSH in moderate COVID-19 patients with respiratory distress.

2. MATERIAL AND METHODS

2.1 Design and Setting

The study was a multicentric, randomized, double-blind, comparative placebo-controlled, Phase III clinical trial to evaluate the efficacy and safety of Intravenous Glutathione, as an add-on to the 'Standard of Care' (SOC) treatment in moderate COVID-19 patients suffering from respiratory distress. After an approval from the Drug Controller General of India, the study was conducted in seven geographically distributed sites across India. The protocol was approved by the institutional ethics committee at each study site.

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The study was performed in accordance with International Council for Harmonization for Good Clinical Practice, Declaration of Helsinki and New Drugs and Clinical Trials, Rules, 2019, The study was registered with the Clinical Trial Registry of India (CTRI/2021/01/030793).

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2.2 Participants

Patients admitted to the hospital were evaluated as per the study eligibility criteria. Patients aged 18 years or older admitted to the hospital with laboratory confirmation of SARS-CoV-2 infection and moderate disease condition as per COVID-19 treatment guideline specified by the Government of India (moderate condition defined as presence of clinical features of dyspnea and/or hypoxia, fever, cough including respiratory rate >24 breaths/min or SpO2 90-94% on room air or pneumonia with no signs of severe disease [31]) were considered eligible.

Asymptomatic COVID-19 patients were excluded. Patients were also excluded if the investigator judged that they had any serious medical conditions and need for invasive or non-invasive ventilator support. All patients or their legally acceptable representatives provided written informed consent to participate in the study. The details of the disposition of patients in the study are given in Figure 2.

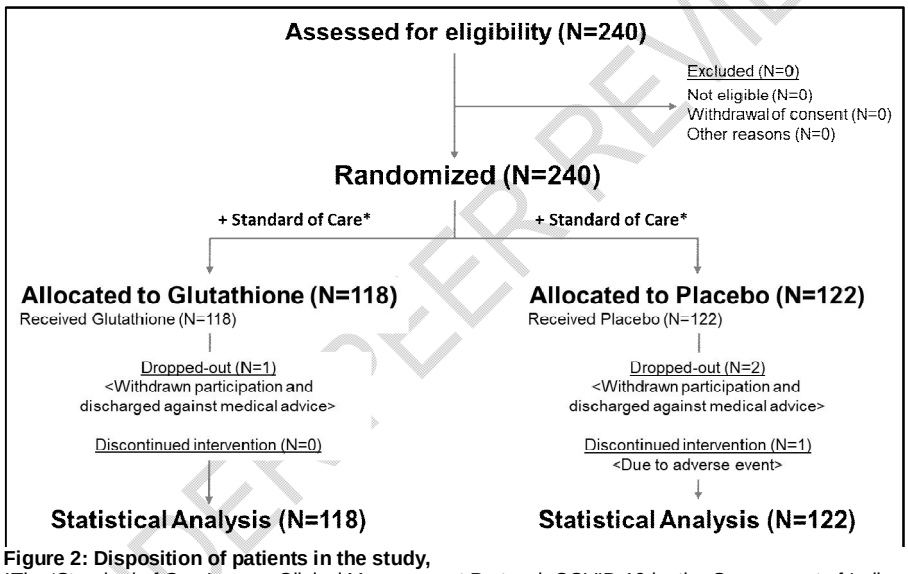

Figure 2: Disposition of patients in the study,
***The 'Standard of Care' as per Clinical Management Protocol: COVID-19 by the Government of India

2.3 Randomization and Blinding

Eligible patients were randomly assigned using block randomization in a ratio of 1:1 to receive GSH plus SOC (GSH group) or placebo plus SOC (Placebo group). Participants from the GSH group received 2400 mg as a loading dose and then 1200 mg every 12-hourly intravenous injection of GSH over 7 days or earlier till the clinical improvement. Since it was a double-blind study, the assigned treatment arm was not known to the site staff, investigator and the patients.

The SOC treatment was administered along with investigational products as per the COVID-19 treatment guidelines specified by the Government of India, in both the treatment groups. SOC included symptomatic treatment, adequate hydration, oxygen support, conservative fluid management, anticoagulation, corticosteroids, anti-viral, control of the co-

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morbid condition and regular monitoring for breathing, hemodynamic stability and oxygen requirement. The SOC was kept as close to the Government treatment protocol as possible in all the study sites.

2.4 Outcome Measures

The clinical status of patients was assessed using the World Health Organization's (WHO) 7-point ordinal scale recommended by the WHO R&D Blueprint Group. [32] Clinical status scores on WHO 7-point ordinal scale were defined as follows: '0': No clinical or virological evidence of infection; '1': No limitation of activities; '2': Limitation of activities '3': Hospitalized, no oxygen therapy; '4': Oxygen by mask or nasal prongs, '5': Non-invasive ventilation or high flow oxygen, '6': Intubation and mechanical ventilation; '7': Ventilation + additional organ support- pressors, receiving renal replacement therapy, extracorporeal membrane oxygenation; '8': Death.

The primary efficacy outcome of the study was a clinical improvement on the WHO 7-point ordinal scale. The clinical improvement was defined as a ≥2-point improvement from the time of enrolment, in disease severity rating on the WHO 7-point ordinal scale. The secondary outcomes were the proportion of patients achieving a score of 3 and below (No Oxygen Requirement) on WHO 7-point ordinal scale, the proportion of patients shifting from higher to a lower score on the WHO 7-Point ordinal scale, the proportion of patients remaining hospitalized, incidences of the need of non-invasive ventilation or new requirement of high flow oxygen use. The outcomes were assessed up to Day 7. Safety was assessed by the number of patients reporting incidences of adverse events (AEs).

2.5 Statistical Analysis

A 40% symptomatic improvement was assumed in patients receiving SOC. A power of 80% with a 5% level of significance was considered to detect at least 60% improvement in patients receiving intravenous glutathione as an add-on to the SOC. Based on the above assumptions, the sample size required per group was found to be 94. Considering dropout or discontinued incidences if any during the study, 240 patients (120 in each group) were randomized in the study.

Descriptive statistics were used to summarize baseline characteristics; data was represented in terms of the number of observations (n), mean ± standard deviation (SD) for continuous variables. Non-continuous data was presented in frequency and percentage. The baseline and demographic characteristics of the two treatment groups were assessed using an unpaired Student's t-test or Pearson-chi^2 test.

The primary endpoint was assessed as the proportion of patients with ≥2 points improvement in each group observed on 7-point WHO ordinal scale using the Pearson-Chi^2 test. The clinical score on the WHO 7-point ordinal scale of two treatment groups was assessed using an unpaired Student's t-test. Relative risk ratio and odds ratio were evaluated for the events of hospitalization in both groups. All analysis results were presented with a significance level of 0.05 and 95% confidence intervals. Safety was summarized descriptively, and AEs and serious adverse events (SAEs) were assessed as the frequency and proportion of patients reporting the event.

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3. RESULTS

3.1 Study Population

During the period February 2021 - September 2021, 240 patients were enrolled and randomized, 118 were assigned to the GSH group and 122 to the placebo group.

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The mean age of the population was 47.03 (range 18-89) years. Patients in both groups were balanced in demographics and disease characteristics. Patients' characteristics are depicted in Table 1 and 2. The prevalence of other comorbidities was equal between groups. Overall, 7.92% of patients had diabetes, and 7.5% had hypertension. All the patients were on supplemental oxygen (on high flow oxygen or on non-invasive ventilation) support at baseline. The oxygen saturation (SpO2) was below 92% at room air and respiratory rate of >26 breaths per min in both the groups at baseline.

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Table 1: Baseline demographics

Demographic Characteristics	GSH + SOC N=118 n (%)	Placebo + SOC N=122 n (%)
Age		
18-40 years	45 (38.14)	46 (37.71)
41-60 years	58 (49.15)	48 (39.34)
≥61 years	15 (12.71)	28 (22.95)
Sex		
Male	81 (68.64)	87 (71.31)
Female	37 (31.36)	35 (28.69)
Clinical Symptoms		
Dyspnoea	113 (95.76)	116 (95.08)
Hypoxia	115 (97.46)	117 (95.90)
Fever	90 (76.27)	104 (85.25)
Cough	116 (98.31)	119 (97.54)
Coexisting conditions		
Chronic Kidney Disease	1 (0.85)	1 (.82)
Diabetes Mellitus	9 (7.63)	10 (8.20)
Hypertension	8 (6.78)	10 (8.20)
Hyperthyroidism	3 (2.54)	6 (4.92)
Asthma/COPD	2 (1.70)	1 (0.82)

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Ischemic Heart Disease	1 (0.85)	3 (2.46)
Obesity (BMI ≥30.0 Kg/m2)	5 (4.24)	4 (3.28)
Heart failure	0	1 (0.82)
Rheumatic Heart Disease	0	1 (0.82)
At least 1 coexisting condition	15 (12.71)	21 (17.13)
>1 coexisting conditions	8 (6.78)	9 (7.38)

Table 2: Baseline clinical characteristics

Clinical Characteristics	GSH + SOC Mean (± SD)	Placebo + SOC Mean (± SD)	p-value*
N	118	122	-
Age, years	45.63 (± 14.42)	48.39 (± 16.44)	0.168
Height, cm	161.82 (± 8.36)	161.87 (± 7.88)	0.967
Weight, Kg	66.5 (± 8.22)	66.56 (± 7.82)	0.952
Body mass index, Kg/m^2	25.43 (± 3.00)	25.42 (± 2.61)	0.956
Pulse Rate, beats/min	76.53 (± 12.18)	77.05 (± 13.55)	0.753
Blood Pressure			
SBP, mmHg	126.21 (± 10.51)	124.93 (± 12.86)	0.397
DBP, mmHg	74.20 (± 10.39)	73.12 (± 10.68)	0.428
SpO2 (%)	91.58 (± 1.39)	91.5 (± 2.02)	0.734
Respiratory Rate, bpm	26.95 (± 3.07)	26.59 (± 1.94)	0.290

** Unpaired t-test*

3.2 Efficacy Assessment

3.2.1 Primary Outcome

According to the WHO recommendation, the participant's clinical improvement was evaluated through an ordinal scale, which measures the severity of the disease over time. Improvement on a WHO 7-point ordinal scale was assessed in terms of patients' clinical status (defined as the reduction of disease severity by 2 or more points), representing a clinically meaningful improvement. The score was recorded daily. Both groups showed a decrease in the scale score indicating improvement over time (Table 3). However, there was a significant clinical improvement in the initiation of the GSH+SOC treatment (p=0.008, day 2). On day 3, GSH+SOC treatment resulted in 2 or more points of improvement on the WHO 7-point Ordinal Scale in 49.15% of the patients as compared to 31.96% on placebo (p=0.007; Pearson-chi^2 test; odds ratio, 2.06; 95% CI, 1.22-3.48). In the subset analysis in patients with a baseline score of 5 or more, a higher proportion of patients treated with GSH (64.63%) showed 2 or more points improvement as compared to the placebo (46.58%)

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(p=0.024; Pearson-chi2 test; odds ratio, 2.10; 95% CI, 1.10-4.00) on the WHO 7-point Ordinal Scale at Day 3. All the patients were treated with SOC; this might be the reason the improvement in clinical status between groups was not statistically significant (p=0.493) on Day 7. However, the odds of improvement by ≥2 points in the ordinal scale were higher and favor the addition of GSH along with SOC treatment (odds ratio 1.25; 95% CI: 0.66-2.37).

Table 3: Number of patients with ≥2-point improvement on the WHO 7-point Ordinal Scale

Study Day	GSH + SOC N (%)	Placebo + SOC N (%)	p-value*	Odds ratio (95% CI)
Day 2	21 (17.80)	8 (6.56)	0.008	3.09 (1.31-7.28)
Day 3	58 (49.15)	39 (31.96)	0.007	2.06 (1.22-3.48)
Day 4	81 (68.64)	73 (59.84)	0.155	1.47 (0.86-2.50)
Day 5	91 (77.12)	90 (73.77)	0.547	1.20 (0.66-2.16)
Day 6	94 (79.66)	93 (76.23)	0.522	1.22 (0.66-2.25)
Day 7	97 (82.20)	96 (78.69)	0.493	1.25 (0.66-2.37)

**Pearson-chi^2 test*
Odds ratios greater than 1 indicate benefit with GSH.

3.2.2 Secondary Outcomes

A higher proportion of Patients in the GSH+SOC group attained a WHO 7-point score of ≤3 (no need of oxygen supplementation) as compared to those in the placebo + SOC group (Table 4). The patients who received GSH as an add-on to the SOC were found to be attaining a score of 3 or below 3 (No need of Oxygen supplementation) in a higher proportion as compared to those in the placebo + SOC group on Day 2 (odds ratio, 1.26; 95% CI, 0.69-2.29), Day 3 (odds ratio, 1.61; 95% CI, 0.97-2.68) and Day 4 (odds ratio, 1.67; 95% CI, 0.89-3.14).

Table 4: Number of patients with scores 3 and below (No Oxygen Supplementation Requirement) on the WHO 7-Point Ordinal Scale

Study Day	GSH + SOC N (%)	Placebo + SOC N (%)	Odds ratio (95% CI)
Day 2	30 (25.42)	26 (20.49)	1.26 (0.69-2.29)
Day 3	70 (59.32)	58 (47.54)	1.61 (0.97-2.68)
Day 4	98 (83.05)	91 (74.59)	1.67 (0.89-3.14)

**Pearson-chi^2 test*
Odds ratios greater than 1 indicate benefit with GSH.

In the subset analysis, patients having a score of 5 or more (at baseline) on the WHO 7-point ordinal scale, the GSH+SOC group had 2 times more patients achieved a score of 3 or below 3 (No need of Oxygen supplementation) as compared to the placebo + SOC group

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viz. 25.61% vs. 12.33%; odds ratio, 2.45; 95% CI, 1.04-5.76 on next day of initiation treatment. The improvement in this subset population was sustained over a period of time and was free of risk of respiratory failure (no oxygen requirement) in GSH as compared to the placebo on Day 3 (64.63% vs.45.21%; odds ratio, 2.06; 95% CI, 1.16-4.23) and Day 4 (89.02% vs. 79.45%; odds ratio, 1.92; 95% CI, 0.86-5.14).

The distribution of clinical status was assessed on the WHO 7-point ordinal scale on Day 1, 2, 3, 4, 5, 6 and 7 after randomization. On day 3, the higher proportion of patients treated with GSH (59.31%) shifted to the mild state where they have not required oxygen as compared to the Placebo group (47.55%). The distribution of clinical status between the GSH and placebo groups was significantly different (p = 0.027 by Wilcoxon rank sum test). Similar distributions of clinical status were observed on Day 4 (p = 0.013 by Wilcoxon rank sum test) and Day 5 (p = 0.022 by Wilcoxon rank sum test) between both treatment groups.

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On further analyzing the subset of patients with baseline score of 5 or more on admission, the higher proportion of patients treated with GSH (25.61%) shifted to the mild state where they were not required oxygen on the next day of treatment (i.e. Day 2) as compared to the Placebo group (12.33%) (p=0.037; Pearson-chi^2 test). The distribution of clinical status on WHO 7-point Ordinal Scale between the GSH and placebo groups was significantly different on Day 2 (p = 0.038 by Wilcoxon rank sum test), Day 3 (p = 0.007 by Wilcoxon rank sum test), Day 4 (p = 0.009 by Wilcoxon rank sum test), Day 5 (p = 0.003 by Wilcoxon rank sum test).

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In all the patients, the risk of the remaining in hospital in the GSH treated group was reduced gradually from the start of treatment compared to the placebo group (Table 5). At the end of the study, 14.75% of patients in the placebo group and 9.32% of patients in the GSH group remained in the hospital (relative risk 0.63; 95% CI: 0.31, 1.28). Adjuvant treatment of GSH reduced the risk of remaining in the hospital by 37% in moderate COVID-19 patients. The median time to discharge from the hospital is 5 days in the GSH group and 6 days in the placebo group.

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Table 5: Number of patients remaining hospitalized

Study Day	GSH + SOC N (%)	Placebo + SOC N (%)	Relative Risk (95% CI)	Risk Reduction in GSH Group relative to placebo
2	117 (99.15)	122 (100)	-	-
3	104 (88.13)	115 (94.26)	0.94 (0.39-2.23)	6%
4	80 (67.80)	95 (77.87)	0.87 (0.57-1.33)	13%
5	54 (45.76)	80 (65.57)	0.70 (0.52-0.94)	30%
6	32 (27.19)	46 (37.71)	0.72 (0.60-0.86)	28%
7	11 (9.32)	18 (14.75)	0.63 (0.58-0.69)	37%

There were 4 (3.28%) patients in the placebo group who required non-invasive ventilation whereas only 2 (1.69%) patients in the GSH group required oxygen supplement for only one day after the start of the treatment. The need for new high-flow oxygen after the start of treatment was much lower in the GSH group. There were 5 (4.10%) patients in the placebo

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group who required new high-flow oxygen whereas only 1 (0.85%) patient in the GSH group required a new oxygen supplement after the start of the treatment.

3.2 Safety Assessment

Safety was evaluated based on the incidences of AEs and SAEs reported during the study. There were 12 AEs and 1 SAE reported during the study. In the GSH + SOC group, 5.08% AEs (vertigo, rashes and headache) were reported in 6 patients, whereas 4.10% AEs were reported in 5 patients in the Placebo+SOC group (hypoxia, vertigo, abdominal pain and rashes). The causality assessment revealed that the AEs may or may not be associated with the investigational drugs as all the patients were receiving SOC along with. All adverse events were of mild to moderate severity and resolved without any sequelae. GSH treatment was well tolerated and the safety was found to be comparable to the Placebo.

One (1) SAE was reported in the study termed as a death in the GSH+SOC group. Respiratory failure was the primary cause of death in COVID-19. The reported SAE (death due to COVID 19 pneumonitis, ARDS, cardiorespiratory arrest with the presence of diabetes mellitus) was not related to the study drug.

4. DISCUSSION

The extensive surface area and blood supply in the lungs are able to provide sufficient oxygen to generate the energy which we need to survive. But this makes the lungs, particularly susceptible to injury due to the relatively high concentration of ROS which is produced by normal metabolism. Beyond this, environmental toxins in the air may cause further injury. Human lungs have evolved complex biochemistry to counter these adverse conditions and GSH is a key player in defense mechanisms. However, with the progression of chronic disease, cellular GSH levels can fall below optimal levels for maintaining good health. Many lung diseases are associated with GSH deficiency. These include ALI, ARDS, asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, chronic bronchitis and various viral and bacterial infections. An exaggerated inflammatory response is also involved during the development of many lung diseases and this is further exacerbated by depleted GSH levels. [20,33]

GSH levels can readily be altered depending on a number of factors including diet and supplements. One therapeutic approach to increasing GSH levels can focus on the administration of exogenous GSH. Exogenous GSH has previously been shown that the increased plasma GSH came mostly from absorption of intact GSH administered through an oral route. [34] This indicates that supplementation is useful to enhance the tissue availability of GSH.

The main risk factors for the more aggressive forms and lethal manifestations of COVID-19 appear exactly in the population that natural or pathological depletion of GSH. [35] Karkhanei *et al* (2020), demonstrate that GSH level as an antioxidant was significantly lower in patients with COVID-19. [36]

In the present study, we have evaluated the effects of intravenous GSH treatment on moderate COVID-19 hospitalized patients with respiratory distress. Among adults with moderate COVID-19, a 7-day course of GSH as an add-on treatment to the SOC, achieved clinically meaningful improvement on the WHO 7-point Ordinal Scale in higher proportion as compared with SOC alone. A notable significant improvement was observed on consecutive 3-day treatment infusions wherein nearly half of the patients from the GSH group showed 2 or more points improvement as compared to the placebo group (p=0.007).

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The addition of GSH to the Standard of Care was associated with more rapid clinical improvement than placebo recipients among patients with COVID-19. GSH also reduced the use of HFNC/NIV or mechanical ventilation compared with patients treated with conventional therapies. GSH also demonstrated better benefit in potentially preventing clinical deterioration for patients whose WHO Ordinal score was 5 or more on admission. GSH was superior to placebo in assessing the odds of improvement in the ordinal scale on day 3 (odds ratio 2.06; 95% CI 1.22-3.48; p = 0.007). It has been suggested that its early use at high doses may become an effective strategy in the treatment of COVID-19 patients.

In 2020, Horowitz *et al*, [28] demonstrated that the use of 2 gm of PO or IV glutathione improved the dyspnea within 1 hour in patients with a history of Lyme and tick-borne co-infections experienced cough and dyspnea and radiological findings consistent with novel coronavirus pneumonia. Repeated use of both 2000 mg of PO and IV glutathione was effective in further relieving respiratory symptoms.

In inflammatory lung diseases, supplementation with exogenous sources of GSH helps to reduce the oxidant content. The oral supplementation of GSH is effective in increasing plasma levels, whereas the IV route increases its levels in the pulmonary epithelial lining fluid in a short period of time. [37] NAC is a precursor of reduced GSH given orally NAC (600mg, bid) significantly decreases the frequency and severity of influenza [38], and reduces the incidences of ventilator-associated pneumonia (VAP) as well. [39] Furthermore, intravenous (IV) NAC treatment (40mg/kg/day) for 3 days in patients with mild-to-moderate acute lung injury, significantly improves systemic oxygenation, reduces the need for ventilatory support and also reduces the mortality rate, [40] suggesting that higher concentrations of GSH are required for potential improvement in clinical outcomes. De Flora *et al* (2020) hypothesized that glutathione supplement could act as a potential therapeutic agent in the treatment of COVID-19 through a variety of potential mechanisms, including scavenging ROS radicals, replenishing intracellular GSH, improving T cell response, and modulating inflammation. [38]

Modulation of the inflammatory process with antioxidants may have a mitigating effect on the development of pneumonia, potentially improving outcomes if high doses (>1200mg) are utilized. Lai *et al* demonstrated that 2400 mg of oral NAC (1200 mg, bid) quickly increased glutathione levels in lymphocytes during chronic inflammatory disease, which was not achieved by a low-dose NAC (600 mg, bid). [41] Another promising study revealed that in ARDS and acute ALI patients, IV NAC at a loading dose of 150 mg/kg on the first day, followed by a dose of 50 mg/kg/day for 3 days, improved oxygenation, and decreased mortality rate compared to control patients. [23]

The present study in moderate COVID-19 patients with respiratory distress revealed that, IV GSH at a loading dose of 2400 mg on the first day, followed by a dose of 1200 mg every 12 hours for seven days, not only improved clinical status (no requiring supplemental oxygen) but also increased the chance of being discharged from the hospital. The distribution of the clinical status on day 5 significantly shifted towards better outcomes in the GSH-treated group.

Since the antiviral effect of glutathione is non-specific, many studies have emphasized the advantages of glutathione in the body, helpful in cytokine storms and cellular injury which are the outcomes of SARS-COV-2 and other viral infections. [28,36,42-45] Therefore, restoration of glutathione levels in COVID-19 patients would be a promising approach for the management of the novel coronavirus SARS-CoV-2. Notably, oral administration of the GSH-precursor has been tested as an effective preventive measure against respiratory viral infections. [29,46] GSH is more bioavailable than NAC. [27]

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Moreover, IV glutathione therapy is effective in relieving dyspnea associated with COVID-19 pneumonia. [28,47] Parenteral injection of reduced glutathione could be an efficient therapy for COVID-19 patients with serious illnesses.

5. CONCLUSION

GSH supplementation may represent a treatment approach for addressing cytokine storm syndrome and respiratory distress in patients with COVID-19 pneumonia. Once patients develop clinically confirmed pneumonia or dyspnea, in addition to regular therapy, supplementation should be given intermittently or continuously to enhance tissue availability of GSH. Due to its favorable safety profile, further exploring the use of GSH in hospitalized patients with COVID-19 for severe pneumonia, in other settings like ARDS eventually with a higher level of evidence with randomized controlled clinical trials.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

CONSENT

The authors declare that written informed consent was obtained from all the patients who participated in this study.

ETHICAL APPROVAL

The study protocol and related documents were approved by the Institutional Ethics Committee at each hospital study center. The authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the Declaration of Helsinki.

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