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

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Authors' contributions

This work was carried out in collaboration between both authors. Author BD designed the study, reviewed and edited the manuscript. Author SS managed the literature searches, performed the statistical analysis and drafted the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This study is aimed at evaluating the efficacy and safety of Intravenous Glutathione 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: Patients were randomized into two groups in a ratio of 1:1, to receive either glutathione or placebo. Both the study drugs were given as an addition to the standard of care (SOC). The study site staff, investigator and patients were blinded to the treatment allocation. The primary endpoint of the study was two or more points of improvement on the WHO 7-point ordinal scale whereas the secondary endpoints were the proportion of patients not requiring oxygen supplementation after treatment. Other secondary endpoints included the proportion of patients

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who changed from a higher to a lower score on the WHO 7-Point ordinal scale, the proportion of patients remaining hospitalized, 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 the GSH group ($p<0.001$) was observed in early treatment days. On day 3, GSH group had improvement in 49.15% of the patients as compared to 31.96% on placebo ($p=0.007$; odds ratio, 2.06; 95% CI, 1.22-3.48). A higher proportion of patients with baseline score of 5 or more in the GSH group (64.63%) showed improvement as compared to the placebo (46.58%) ($p=0.024$; odds ratio, 2.10; 95% CI, 1.10-4.00). A higher proportion of patients in the GSH group attained a score of 3 or less signifying no need of Oxygen supplementation, as compared to the placebo group on Day 2, Day 3 and Day 4. A reduction of severity in clinical status was also observed on Day 3, Day 4 and Day 5. The risk of remaining in the hospital was reduced by 37% in the GSH group. The 7-day dose of GSH was well tolerated by the patients.

Conclusion: GSH supplementation reduces the cytokine storm and respiratory distress in patients with COVID-19 pneumonia. GSH can also be used for treating respiratory distress due to other etiologies due to its favorable safety profile. GSH treatment should also be explored in a larger number of patients with ARDS of varied etiologies in randomized trials.

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 that causes diffuse alveolar damage that results 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].

"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].

"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 lung microcirculation. 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].

"A dominant role in the pathogenesis of ALI/ARDS is an oxidative injury to the lung mediated by reactive oxygen species (ROS) 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 that amplify tissue damage and pulmonary edema" [13].

Proper oxidant-antioxidant balance is critical for vasculature homeostasis and the systems responsible for excessive ROS production can be therapeutic targets in ALI/ARDS treatment.

"Glutathione (GSH) is the most abundant antioxidant and a major detoxification agent in 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 that require 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 GSH depletion and contribute to a condition related to 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.

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

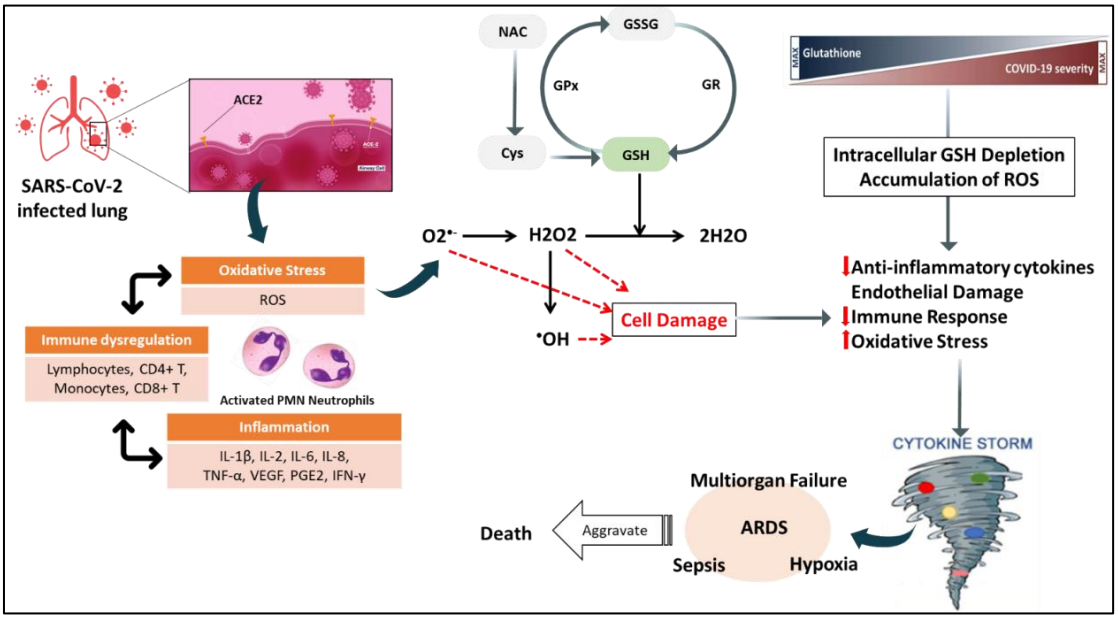

Fig. 1. Schematic representation of GSH against COVID-19 cytokine storm and its associated risk

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

inflammatory response that increases the severity of COVID-19 [24]. In particular, the severity or mortality of the disease is due to cytokine storms triggered by viral lung infection, which contributes to multi-organ failure across the body [25]. Despite recent advances in understanding the mechanism and treatment of COVID-19-related ARDS, its incidence and mortality rate remain high in the inflammatory phase of COVID-19 [26]. Polonikov (2020), studied four moderate-severe cases of COVID-19 and found that the three patients with normal/high plasma levels of GSH recovered rapidly, the one with low levels of GSH, high plasma ROS and ROS/GSH ratio exacerbated the COVID-19 disease [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]. Therefore, 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 Fig. 1.

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 intravenous glutathione. Taking into account 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 predominantly reduced form of GSH in patients with moderate COVID-19 with respiratory distress.

2. MATERIALS 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 addition to the 'standard of care' (SOC) treatment in moderate COVID-19 patients suffering from respiratory distress. After approval from the Drug Controller General of India, the study was conducted in seven geographically distributed sites throughout India. The protocol was approved by the institutional ethics committee at each study site.

The study was carried out according to the 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).

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 noninvasive 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 Fig. 2.

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 in the GSH group received 2400 mg as loading dose and then 1200 mg every 12-hour 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 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, antiviral, control of the comorbid condition and regular monitoring of breathing,

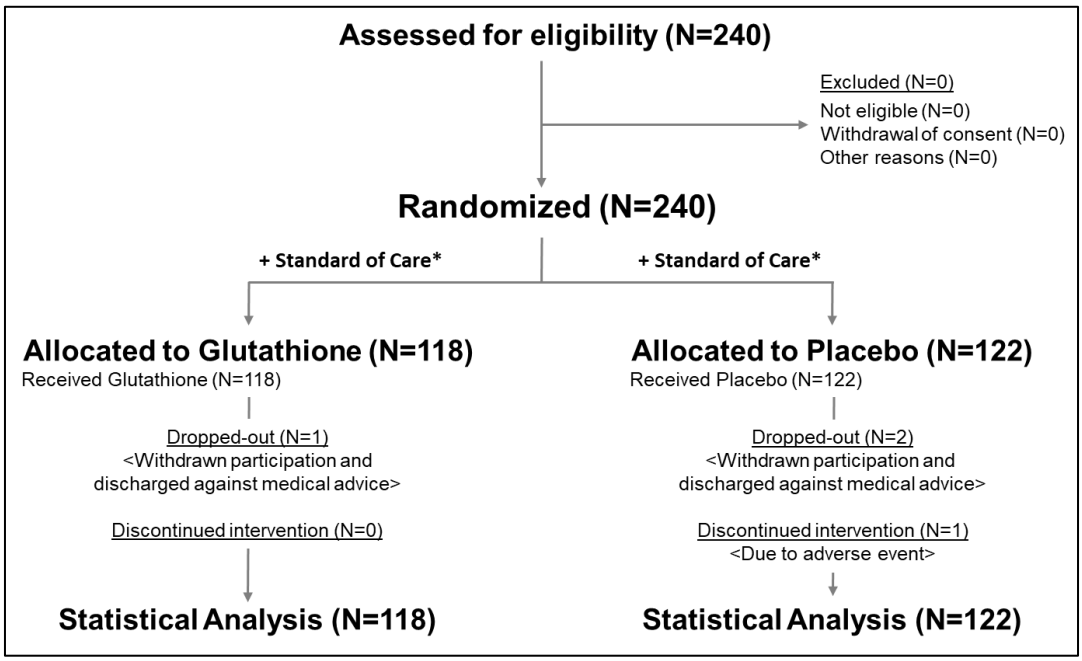

Fig. 2. Disposition of patients in the study

The 'Standard of Care' as per Clinical Management Protocol: COVID-19 by the Government of India

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% significance level was considered to detect at least a 60% improvement in patients who received intravenous glutathione as an addition 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 were 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 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. The relative risk ratio and the odds ratio for hospitalization events were evaluated 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.

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.

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 Tables 1 and 2. The prevalence of other comorbidities was equal between groups. In general, 7.92% of the patients had diabetes, and 7.5% had hypertension. All patients were on supplemental oxygen (on high flow oxygen or on noninvasive 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.

3.2 Efficacy Assessment

3.2.1 Primary outcome

According to the WHO recommendation, the clinical improvement of participants was evaluated using an ordinal scale, which measures the severity of the disease over time. Improvement was assessed in terms of the patient's clinical status (defined as the reduction in 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

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 (0.82)
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)
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*

score indicating improvement over time (Table 3). However, there was a significant clinical improvement in the initiation of 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%) (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 patients were treated with SOC; this could be the reason that improvement in clinical status between groups was not statistically significant (p=0.493) on Day 7. However, the odds of improvement by 2 points on 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).

3.2.2 Secondary outcomes

A higher proportion of patients in the GSH+SOC group achieved a WHO 7-point score of ≤3 (no need for oxygen supplementation) as compared to those in the placebo + SOC group (Table 4; Fig. 3). The patients who received GSH as an add-on to the SOC were found to be attaining a score of 3 or below 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).

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 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.

On further analysis of the subset of patients with a baseline score of 5 or more on admission, the highest 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).

The mean WHO score 3 or below was achieved in the GSH group on day 3 whereas on day 4 in the placebo group (Fig. 4). The mean WHO score on Day 3 (*P*=0.012) and Day 4 (*P*=0.025) in the GSH group was significant as compared to the placebo group.

Similarly, the subset of patients with baseline score 5 and above of the GSH group significantly attained the mean WHO score of less than 3 on Day 3 (*P*=0.005) and on Day 4 (*P*=0.017) compared to the placebo group (Fig. 5).

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 ratio greater than 1 indicates benefit with GSH

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 ratio greater than 1 indicates benefit with GSH

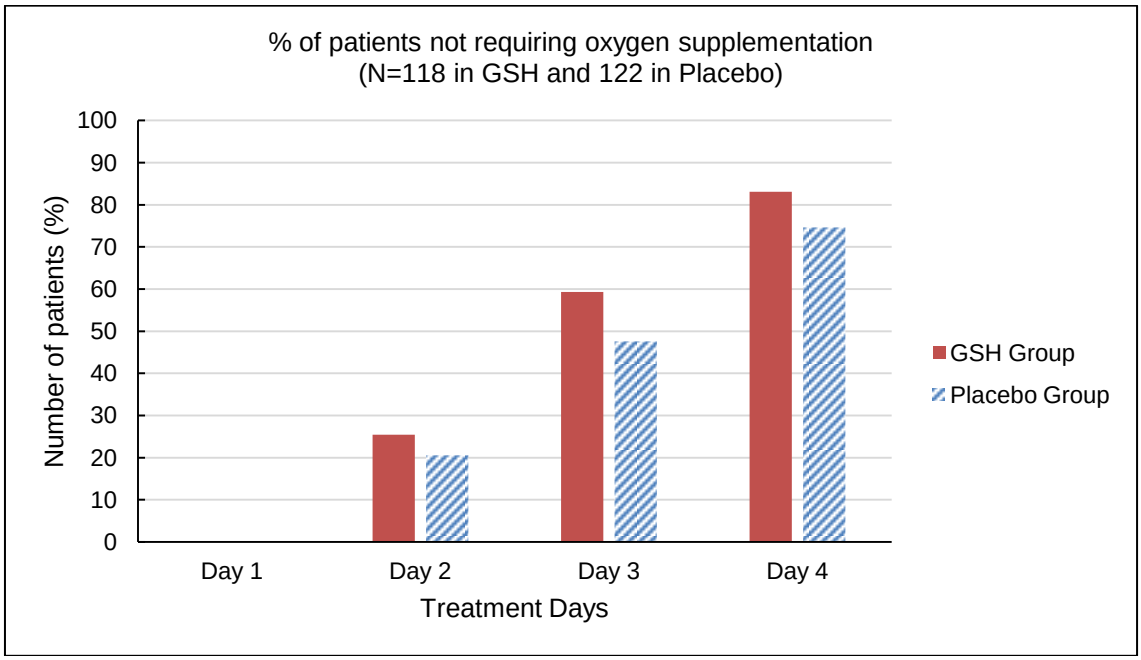

Fig. 3. Number of patients (%) not requiring oxygen supplementation

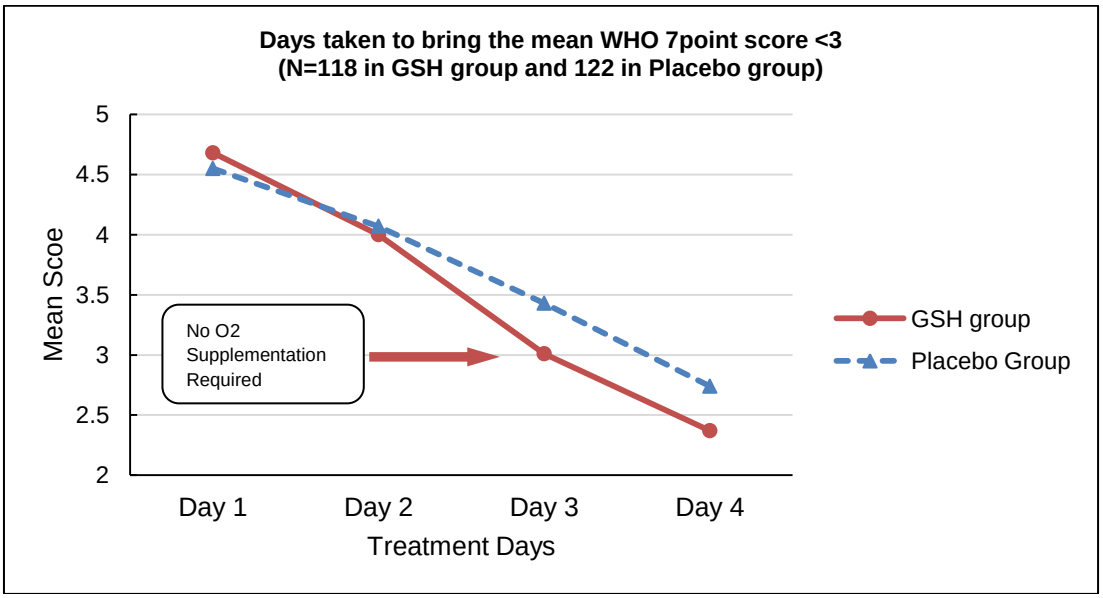

Fig. 4. Number of days taken to bring the mean WHO score <3

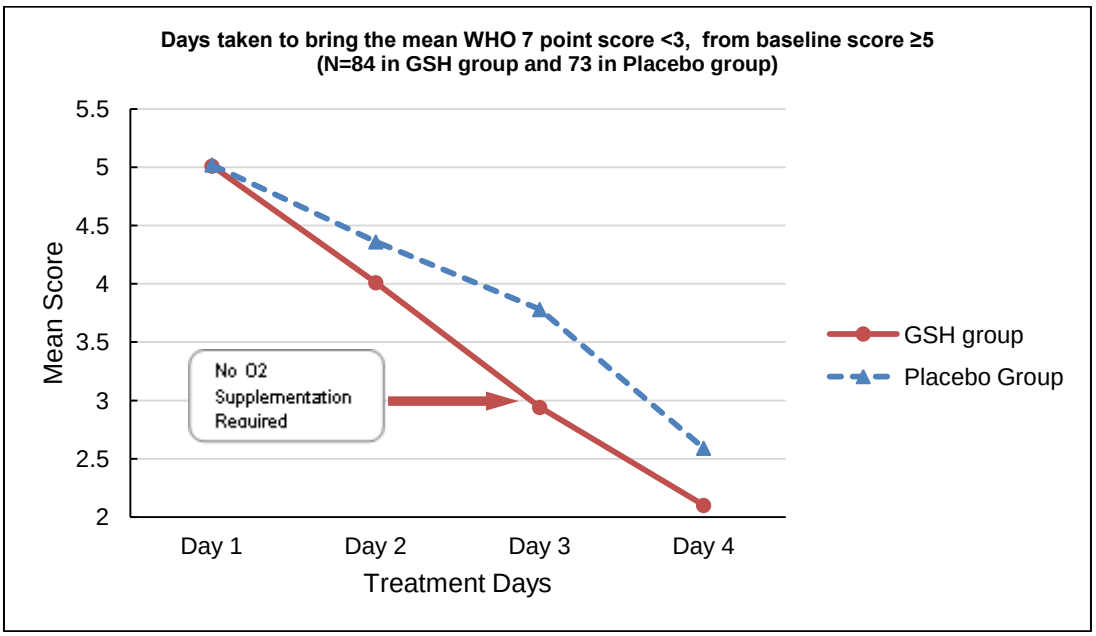

Fig. 5. Number of days taken to bring the mean WHO score 3 or below in the subset of patients with baseline score ≥5

In all patients, the risk of remaining in hospital in the GSH-treated group was gradually reduced 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 with GSH reduced the risk of remaining in the hospital by 37% in patients with moderate COVID-19. The median time to discharge from the hospital is 5 days in the GSH group and 6 days in the placebo group.

There were 4 (3.28%) patients in the placebo group who required noninvasive ventilation while only 2 (1.69%) patients in the GSH group required an oxygen supplement for only one day after the start of 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 group who required new high-flow oxygen while only 1 (0.85%) patient in the GSH group required a new oxygen supplement after the start of treatment.

Table 5. Number of patients who remain 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%

3.3 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 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 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 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.

In the study, one (1) SAE was reported 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 can 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. A therapeutic approach to increasing GSH levels can focus on the administration of exogenous GSH. Exogenous GSH has previously been shown that increased plasma GSH came mainly from absorption of intact GSH administered via 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. demonstrate that the level of GSH 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 in 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 significant improvement was observed on consecutive 3-day treatment infusions, in which nearly half of the patients in the GSH group showed a 2 or more points improvement compared to the placebo group ($p=0.007$). The addition of GSH to the standard of care was associated with more rapid clinical improvement than placebo recipients among COVID-19 patients. 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 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. Oral GSH supplementation 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 patients with ARDS and acute ALI, 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 changed significantly 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 to the treatment of the new 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]. 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 administered intermittently or continuously to improve the tissue availability of GSH. Due to its favorable safety profile, further exploration of 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.

CONSENT

The authors declare that written informed consent was obtained from all 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dushianthan A, Grocott MP, Postle AD, Cusack R. Acute respiratory distress syndrome and acute lung injury. Postgrad Med J. 2011;87(1031):612-22.
DOI: 10.1136/pgmj.2011.118398
2. Johnson ER, Matthay MA. Acute lung injury: Epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv. 2010;23(4):243-52.
DOI: 10.1089/jamp.2009.0775
3. Parekh D, Dancer RC, Thickett DR. Acute lung injury. Clin Med. 2011;11(6):615-8.
DOI: 10.7861/clinmedicine.11-6-615
4. Malek R, Soufi S. Pulmonary Edema. [Updated 2021 Apr 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.
Available:https://www.ncbi.nlm.nih.gov/books/NBK557611/
5. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, et al. Incidence and outcomes of acute lung injury. N Engl J Med. 2005;353(16):1685-93.
DOI: 10.1056/NEJMoa050333
6. Singh G, Gladdy G, Chandy TT, Sen N. Incidence and outcome of acute lung injury and acute respiratory distress syndrome in the surgical intensive care unit. Indian J Crit Care Med. 2014; 18(10):659-65.
DOI: 10.4103/0972-5229.142175
7. Kinnula VL, Crapo JD. Superoxide dismutases in the lung and human lung diseases. Am J Respir Crit Care Med. 2003;167(12):1600-19.
DOI: 10.1164/rccm.200212-1479SO
8. Büyükbaş S, Uzun K, Demirkapı E, Başaralı K. Oxidative stress and antioxidant status in bronchoalveolar lavage fluid, plasma and erythrocyte of critically mixed ill with respiratory failure. European Journal of General Medicine. 2008;5(3):140-6.
9. Lee WL, Downey GP. Neutrophil activation and acute lung injury. Curr Opin Crit Care. 2001;7(1):1-7.
DOI: 10.1097/00075198-200102000-00001
10. Najafi A, Mojtahedzadeh M, Mahmoodpoor A, Aghamohammadi M, Ahmadi A, Nahreini S, *et al*. Effect of N-acetylcysteine on microalbuminuria in patients with acute respiratory distress syndrome. Arch Med Sci. 2009;5(3):408-14.
11. Schmidt R, Luboeinski T, Markart P, Ruppert C, Daum C, Grimminger F, *et al*. Alveolar antioxidant status in patients with acute respiratory distress syndrome. Eur Respir J. 2004;24(6):994-9.
DOI: 10.1183/09031936.04.00120703
12. Bellingan GJ. The pulmonary physician in critical care * 6: The pathogenesis of ALI/ARDS. Thorax. 2002;57(6):540-6.
DOI: 10.1136/thorax.57.6.540
13. Kellner M, Noonepalle S, Lu Q, Srivastava A, Zemskov E, Black SM. ROS Signaling in the Pathogenesis of Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS). Adv Exp Med Biol. 2017;967:105-137.
DOI: 10.1007/978-3-319-63245-2_8.
14. Lin S, Wu H, Wang C, Xiao Z, Xu F. Regulatory T cells and acute lung injury: Cytokines, uncontrolled inflammation, and therapeutic implications. Front Immunol. 2018;9:1545
DOI: 10.3389/fimmu.2018.01545.
15. Moldoveanu B, Otmishi P, Jani P, Walker J, Sarmiento X, Guardiola J, et al.

Inflammatory mechanisms in the lung. J Inflamm Res. 2009;2:1-11.
PMID: 22096348

16. Cross LM, Matthay MA. Biomarkers in acute lung injury: insights into the pathogenesis of acute lung injury. Crit Care Clin. 2011;27(2):355-77.
DOI: 10.1016/j.ccc.2010.12.005
17. Sarkele M, Sabelnikovs O, Vanags I, Ozolina A, Skesters A, Silova A. The role of oxidative stress markers in acute respiratory distress syndrome. Acta Chirurgica Latviensis. 2013;13(2):22-6.
DOI: 10.2478/chilat-2014-0005
18. Pizzorno J. Glutathione. Integr Med. 2014; 13(1):8.
PMID: 26770075
19. Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal. 2009;11(11):2685-700.
DOI: 10.1089/ARS.2009.2695
20. Ghezzi P. Role of glutathione in immunity and inflammation in the lung. Int J Gen Med. 2011;4:105-13.
DOI: 10.2147/IJGM.S15618
21. Aquilano K, Baldelli S, Ciriolo MR. Glutathione: New roles in redox signaling for an old antioxidant. Front Pharmacol. 2014;5:196.
DOI: 10.3389/fphar.2014.00196
22. Basi Z, Turkoglu V. In vitro effect of oxidized and reduced glutathione peptides on angiotensin converting enzyme purified from human plasma. Journal of Chromatography B. 2019;1104:190-5.
DOI: 10.1016/j.jchromb.2018.11.023
23. Moradi M, Mojtahedzadeh M, Mandegari A, Soltan-Sharifi MS, Najafi A, Khajavi MR, et al. The role of glutathione-S-transferase polymorphisms on clinical outcome of ALI/ARDS patient treated with N-acetylcysteine. Respir Med. 2009;103(3): 434-41.
DOI: 10.1016/j.rmed.2008.09.013
24. Derouiche S. Oxidative stress associated with SARS-Cov-2 (COVID-19) increases the severity of the lung disease-a systematic review. J Infect Dis Epidemiol. 2020;6(121):10-23937.
DOI: 10.23937/2474-3658/1510121
25. Hojyo S, Uchida M, Tanaka K, Hasebe R, Tanaka Y, Murakami M, et al. How COVID-19 induces cytokine storm with high mortality. Inflamm Regen. 2020;40(1):1-7.
DOI: 10.1186/s41232-020-00146-3
26. Acosta MA, Singer BD. Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. Eur Respir J. 2020;56(3).
DOI: 10.1183/13993003.02049-2020
27. Polonikov A. Endogenous deficiency of glutathione as the most likely cause of serious manifestations and death in COVID-19 patients. ACS Infect Dis. 2020; 6(7):1558-62.
DOI: 10.1021/acsinfecdis.0c00288
28. Horowitz RI, Freeman PR, Bruzzese J. Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: A report of 2 cases. Resp MedCase Resp. 2020;30:101063.
DOI: 10.1016/j.rmcr.2020.101063
29. Zhang Q, Ju Y, Ma Y, Wang T. N-acetylcysteine improves oxidative stress and inflammatory response in patients with community acquired pneumonia: A randomized controlled trial. Medicine. 2018;97(45):e13087.
DOI: 10.1097/MD.0000000000013087
30. Bernard GR, Wheeler AP, Arons MM, Morris PE, Paz HL, Russell JA, *et al*. A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The Antioxidant in ARDS Study Group Chest. 1997;112(1): 164-72.
DOI: 10.1378/chest.112.1.164
31. Ministry of Health and Family Welfare, Directorate General of Health Services, EMR Division. Guidance document on appropriate management of suspect/confirmed cases of COVID-19; July 3, 2020.
32. WHO R&D blueprint novel Coronavirus COVID-19 Therapeutic Trial Synopsis February 18, 2020, Geneva, Switzerland.
33. Gould NS, Day BJ. Targeting maladaptive glutathione responses in lung disease. Biochem Pharmacol. 2011;81(2):187-93.
DOI: 10.1016/j.bcp.2010.10.001
34. Hagen TM, Wierzbicka GT, Sillau AH, Bowman BB, Jones DP. Bioavailability of dietary glutathione: efFect on plasma concentration. Am J Physiol. 1990; 259(4):G524-9.
DOI: 10.1152/ajpgi.1990.259.4.G524
35. Setti TM, Setti TS, da Fonseca LF, Huber SC, Santos GS, Lana J. The role of Glutathione as an adjunct therapy in the treatment of patients with COVID-19-Related Acute Respiratory Syndrome. Advance Research Journal of Medical and Clinical Science. 2021;7(2),415-427.

DOI: 10.15520/arjmcs.v7i02.247
36. Karkhanei B, Ghane ET, Mehri F. Evaluation of oxidative stress level: Total antioxidant capacity, total oxidant status and glutathione activity in patients with COVID-19. New Microbes New Infect. 2021;42:100897.
DOI: 10.1016/j.nmni.2021.100897
37. Lana JF, Lana AV, Rodrigues QS, Santos GS, Navani R, Navani A, et al. Nebulization of glutathione and N-Acetylcysteine as an adjuvant therapy for COVID-19 onset. Advances in Redox Research. 2021;3:100015.
DOI: 10.1016/j.arres.2021.100015
38. De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. Eur Respir J. 1997;10(7):1535-41.
DOI: 10.1183/09031936.97.10071535
39. Sharafkhah M, Abdolrazaghnejad A, Zarinfar N, Mohammadbeigi A, Massoudifar A, Abaszadeh S. Safety and efficacy of N-acetyl-cysteine for prophylaxis of ventilator-associated pneumonia: A randomized, double blind, placebo-controlled clinical trial. Med Gas Res. 2018 Jan;8(1):19.
DOI: 10.4103/2045-9912.229599
40. Suter PM, Domenighetti G, Schaller MD, Laverrière MC, Ritz R, Perret C. N-acetylcysteine enhances recovery from acute lung injury in man: A randomized, double-blind, placebo-controlled clinical study. Chest. 1994 Jan 1;105(1):190-4.
DOI: 10.1378/chest.105.1.190
41. Lai ZW, Hanczko R, Bonilla E, Caza TN, Clair B, Bartos A, et al. N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: A randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 2012;64(9):2937–2946.
DOI: 10.1002/art.34502
42. Muhammad Y, Kani YA, Iliya S, Muhammad JB, Binji A, El-Fulaty Ahmad A, et al. Deficiency of antioxidants and increased oxidative stress in COVID-19 patients: A cross-sectional comparative study in Jigawa, Northwestern Nigeria. SAGE open medicine. 2021 Jan;9: 2050312121991246.
DOI: 10.1177/2050312121991246
43. Guloyan V, Oganesian B, Baghdasaryan N, Yeh C, Singh M, Guilford F, et al. Glutathione supplementation as an adjunctive therapy in COVID-19. Antioxidants. 2020 Oct;9(10):914.
DOI: 10.3390/antiox9100914
44. Khanfar A, Al Qaroot B. Could glutathione depletion be the Trojan horse of COVID-19 mortality. Eur Rev Med Pharmacol Sci. 2020;24(23):12500-9.
DOI: 10.26355/eurrev_202012_24046
45. Silvagno F, Vernone A, Pescarmona GP. The role of glutathione in protecting against the severe inflammatory response triggered by COVID-19. Antioxidants. 2020 Jul;9(7):624.
DOI: 10.3390/antiox9070624
46. Izquierdo JL, Soriano JB, González Y, Lumbreras S, Ancochea J, Echeverry C, Rodríguez JM. Use of N-Acetylcysteine at high doses as an oral treatment for patients hospitalized with COVID-19. Science Progress. 2022;105(1):1-12.
DOI: 10.1177/00368504221074574
47. Shi Z, Puyo CA. N-acetylcysteine to combat COVID-19: an evidence review. Therapeutics and Clinical Risk Management. 2020;16:1047-55.
DOI: 10.2147/TCRM.S27370

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