Abstract. – Since November 2019, SARS Coronavirus 2 disease (COVID-19) pandemic has spread through more than 195 nations worldwide. Though the coronavirus infection affects all age and sex groups, the mortality is skewed towards the elderly population and the cause of death is mostly acute respiratory distress syndrome (ARDS). There are data suggesting the role of excessive immune activation and cytokine storm as the cause of lung injury in COVID-19. The excessive immune activation and cytokine storm usually occurs due to an imbalance in redox homeostasis of the individuals. Considering the antioxidant and free radical scavenging action of N-acetyl cysteine (NAC), its use might be useful in COVID-19 patients by decreasing the cytokine storm consequently decreasing the disease severity. Therefore, we reviewed all the available resources pertaining to the role of reactive oxygen species (ROS) in cytokine storm and the mechanism of action of NAC in preventing ROS. We also reviewed the use of NAC in COVID-19.

Key Words: Immune activation, Reactive oxygen species (ROS), Oxidative stress, Redox homeostasis, Free radicals.

Introduction

Since November 2019, SARS Coronavirus 2 disease (COVID-19) pandemic has spread through more than 195 nations worldwide. According to the world health organization (WHO) coronavirus disease (COVID-19) dashboard, approximately 20 million people were infected and 0.7 million succumbed to death till August 15, 2020. Though the coronavirus infection affects all age and sex groups, the mortality is skewed towards the elderly population and the cause of death is mostly acute respiratory distress syndrome (ARDS). There are data suggesting the role of excessive immune activation and cytokine storm as the cause of lung injury in COVID-19. The excessive immune activation and cytokine storm usually occurs due to an imbalance in redox homeostasis of the individuals. Considering the antioxidant and free radical scavenging action of N-acetyl cysteine (NAC), its use might be useful in COVID-19 patients by decreasing the cytokine storm consequently decreasing the disease severity. Therefore, we reviewed all the available resources pertaining to the role of reactive oxygen species (ROS) in cytokine storm and the mechanism of action of NAC in preventing ROS. We also reviewed the use of NAC in COVID-19.

Key Words: Immune activation, Reactive oxygen species (ROS), Oxidative stress, Redox homeostasis, Free radicals.

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NAC as a treatment option in COVID-19, it is being used as an off-label treatment at many centers. Therefore, we searched all the available resources in PubMed, Cochrane library on the use of NAC to review two key objectives, i.e.,

Therapeutic potential of NAC in decreasing the immune activation and cytokine storm.

Efficacy of NAC in management of COVID-19.
We also reviewed all the studies registered in clinicaltrial.gov on the use of NAC in COVID-19.

Reactive Oxygen Species Induced Cytokine Storm
Excessive production of ROS due to oxidative stress and decreased antioxidant level plays a central role in the development of cytokine storm during infections. Many predisposing factors like diabetes, inflamming, decrease glutathione level with aging triggers ROS production, and results in cytokine storm due to increased oxidative stress. ROS causes cell death by apoptosis and increases the release of pro-inflammatory cytokines like IL-2, IL-6, and TNF-α by activated macrophages. The rise of IL-2 and IL-6 has been well documented in COVID-19 in the hyper-inflammatory stage of the disease. There is a decrease in CD4, CD8 lymphocytes and increase neutrophil to lymphocyte ratio (NLR) in severe COVID 19 along with a rise in monocyte macrophages leading to a rise in pro-inflammatory cytokines IL-6, IL-2. This hyper-inflammatory immune response leads to the production of ROS by epithelial cells. ROS stimulates the synthesis of (NOD)-like receptor protein 3 inflammasome; (NLRP3) and nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB) which propages the cytokine production. These pro-inflammatory cytokines increase the release of nitric oxide (NO) which causes vasodilatation, hypotension, vascular leakage, and organ damage. Thus, in the latter part of COVID-19, these cytokines and free radicals play a major role in disease pathogenesis rather than the virus itself.

NAC as an Antioxidant
N-acetyl cysteine (NAC) is a safe and inexpensive medication commercially available since 1960. It is the N-acetyl derivative of the natural amino acid L-cysteine and has been used in a variety of conditions including lung diseases. However, only USFDA approved indication of NAC is as an antidote for paracetamol poisoning. Many studies in humans have demonstrated the antioxidant property of NAC. The antioxidant property of NAC can be attributed to three mechanisms a) direct antioxidant activity b) indirect antioxidant activity, and c) disulfide bond-breaking activity. The direct antioxidant activity of NAC against reactive oxygen species like H₂O₂, ONOOH, O₂⁻, HO, HO(X), and NO₂ due to the presence of thiol group in it has been established in both in vitro and in vivo studies. NAC has highest antioxidant activity towards NO and HO(X) in vivo. It exhibits indirect antioxidant activity by increasing the glutathione level at the site of inflammation. Meyer et al demonstrated that NAC increased the level of glutathione to almost normal levels in lung fluid in patients with pulmonary fibrosis. NAC also exerts mucolytic activity in the lungs in inflammatory conditions by its disulfide bond-breaking action. The breakdown products increase free thiol which exhibits antioxidant action by directly inhibiting the reactive oxygen species (ROS) and indirectly by increasing the glutathione level. The pathogenesis of the cytokine storm and the action of NAC has been depicted in Figure 1.

Therapeutic Potential of NAC in Decreasing the Immune Activation and Cytokine Storm
N-acetyl cysteine has been used as an antioxidant in clinical practice for decades, but its uses are mostly off-label. However, there is ample evidence in preclinical and clinical studies that it can attenuate immune activation and prevent cytokine release. Davreux et al demonstrated that lipopolysaccharide-induced increased lung permeability and lung injury is significantly attenuated by NAC in a rat model. In the same study, although NAC did not affect the migration of neutrophils to the site of injury, it significantly decreased the activation and cytokine production by neutrophils. In another study by Ungheri et al, NAC significantly decreased the mortality in influenza affected mice by decreasing the production of ROS and cytokines like TNF and IL-6. Similarly, Ergin et al and Garozzo et al demonstrated the beneficial effect of NAC in influenza and acute kidney injury in the rat model. Animal studies showing the antioxidant effect of NAC in inflammation depicted in Table I.

From the above studies, it is evident that NAC has therapeutic potential in decreasing the immune activation and resultant cytokine storm.
The clinical utility of NAC in various diseases has been proved by many studies\(^8-11\). Study characteristics and its outcome of NAC use in different lung diseases in humans are depicted in Table II.

The addition of NAC to the standard treatment protocol decreases the severity of many acute respiratory conditions like influenza, community-acquired pneumonia (CAP), ARDS, ventilator-associated pneumonia (VAP) mostly by atten-

**Table I.** Animal studies showing the antioxidant effect of NAC.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study hypothesis</th>
<th>Observation</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davreux et al(^27) 1997</td>
<td>NAC might attenuate lung injury following an intratracheal challenge with endotoxin (lipopolysaccharide; LPS)</td>
<td>Significant decrease in lung permeability due to lipopolysaccharide (LPS) induced lung injury in mice (p &lt; 0.5)</td>
<td>Scavenging of free radicals and decrease in the neutrophil oxidative burst</td>
</tr>
<tr>
<td>Ungheri et al(^28) 2000</td>
<td>NAC may decrease the level of ROS in mice infected intranasally by influenza virus</td>
<td>Administration of NAC in influenza infected mice decreases mortality significantly</td>
<td>Decreasing ROS (XO, TNF, IL-6)</td>
</tr>
<tr>
<td>Garozzo et al(^29) 2007</td>
<td>Addition of NAC to oseltamivir decreases the oxidative stress in viral infection in murine model</td>
<td>Combining NAC with oseltamivir increases the survival of mice</td>
<td>Antioxidant effect against oxidative stress associated with viral infection</td>
</tr>
<tr>
<td>Ergin et al(^12) 2016</td>
<td>Improvement in microcirculatory oxygenation in kidney may occur due to addition of NAC along with fluid resuscitation by reduction of oxidative stress.</td>
<td>NAC supplementation in resuscitation fluids improved renal microcirculatory oxygenation, inflammation, and function in a rat model of endotoxemia</td>
<td>Dampening the accumulation of NO in kidney tissue (p &lt; 0.01)</td>
</tr>
</tbody>
</table>
Therapeutic potential of NAC in preventing cytokine storm in COVID-19

Evaluating the immune activation. In all the studies, the daily dose of NAC, 1200 mg, was well tolerated without any significant adverse reaction. Though there is limited direct evidence, it can be inferred from these studies that the clinical efficacy of NAC is possibly due to the composite effect of its antioxidant and immunomodulatory activities.

**Efficacy of NAC in Management of COVID-19**

As the role of ROS and cytokine storm in the pathogenesis of COVID-19 became more evident, many hypotheses argued in favor of N-acetyl cysteine use in COVID-19. Nasi et al. suggested evaluation of NAC as a cost-effective therapeutic measure in COVID-19 for protection against excessive ROS production. Further, Andreou et al. hypothesized that the combination of remdesivir, copper, NAC, NO, and colchicine could be an effective treatment option for COVID-19.

Till the end of July 2020, robust published data regarding the indication, dosing, safety, and efficacy of NAC in COVID-19 was lacking. We found only 2 case series related to the use of NAC in COVID-19. The details of the studies are depicted in Table III.

In both the case series, all patients improved. Ibrahim et al. documented that there is a decrease in serum CRP and ferritin levels in all patients after NAC therapy indicating the possibility of a reduction in oxidative stress. There was a significant improvement in liver function and oxygen requirement in all patients after the addition of NAC. Nine patients requiring ECMO were improved and discontinued from ECMO after initiation of NAC. Though the outcome is encouraging, it is difficult to predict the efficacy of NAC in COVID-19 as the sample size is extremely small, and also all the patients were receiving multiple combinations of therapies.

Presently there are seven ongoing clinical trials evaluating the dose, safety, and efficacy of NAC therapy in COVID-19.

The outcome measures in different clinical trials range from clinical improvement, safety and tolerability, change in lung function to change in inflammatory cytokines. The results of these trials in the near future shall clarify the utility of this agent in COVID-19.

**Conclusions**

Shortly, NAC has proven antioxidant activity which is useful in attenuating the immune activation and cytokine storm in animal and human studies. It is a safe, cost-effective, widely available drug, and its mechanism of action hypothetically suggests its potential role in the manage-

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**Table II. Use of NAC in different lung diseases in human.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Title</th>
<th>Study design</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flora et al. 1997</td>
<td>“Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment”</td>
<td>Randomized control trial</td>
<td>NAC treatment decreased the severity of infection and increased cell mediated immunity.</td>
</tr>
<tr>
<td>Zhang et al. 2018</td>
<td>“N-acetyl cysteine improves oxidative stress and inflammatory response in patients with community acquired pneumonia”</td>
<td>Randomized control trial</td>
<td>Significant decrease in TNF-α (p &lt; 0.0010) in NAC group along with increase in total antioxidant capacity.</td>
</tr>
<tr>
<td>Zhang et al. 2017</td>
<td>Effects of N-acetylcysteine treatment in acute respiratory distress syndrome: a meta-analysis</td>
<td>Meta-analysis</td>
<td>NAC group had less duration of intensive care unit stay.</td>
</tr>
<tr>
<td>Sharafkhah et al. 2018</td>
<td>“Safety and efficacy of N-acetyl-cysteine for prophylaxis of ventilator-associated pneumonia: a randomized, double blind, placebo-controlled clinical trial”</td>
<td>Randomized control trial</td>
<td>Incidence of less severe disease and complete recovery was significantly higher in NAC treated group. The suggested mechanism for improvement is due to antioxidant an mucolytic action of NAC.</td>
</tr>
</tbody>
</table>


Therapeutic potential of NAC in preventing cytokine storm in COVID-19


