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High dose vitamin c improves inflammatory markers and clinical outcome of patients with acute respiratory distress syndrome

Nada H. Farrag[a], Lamia M. El Wakeel[b*], Ahmed M. Abdelhafeez[c], Mona F. Schaalan[d]

[a]*Department of Clinical Pharmacy, School of Pharmacy, New Giza University, Cairo, Egypt*
[b]*Department of Clinical Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*
[c]*Department of Chest Diseases, Faculty of Medicine, Cairo University, Cairo, Egypt*
[d]*Department of Clinical Pharmacy, Faculty of Pharmacy, Misr International University, Cairo, Egypt*

ABSTRACT

To assess the efficacy, tolerability, and clinical outcome of high dose IV Vitamin C administration in patients suffering from acute respiratory distress syndrome (ARDS). A prospective, randomized, controlled, open-label study was conducted at the Intensive Care Unit of the National Center for Allergy and Chest Diseases, Cairo, Egypt. Forty clinically and radiologically diagnosed cases of eligible ARDS patients were randomized to either, Group 1 (Control); 20 patients received conventional ARDS management, or Group 2 (Test); 20 ARDS patients received 10 g IV Vitamin C on two divided doses, both for 10 days. Vitamin C, Interleukin 8 (IL8), and nuclear factor erythroid 2–related factor 2 (NRf2) levels together with PaO_2/FiO_2 were all measured for both groups at baseline and after 10 days from study start. Both groups were comparable at baseline. After 10 days of Vitamin C administration, a significant increase (P<0.001) in levels of Vitamin C, NRf2, and PaO_2/FiO_2 together with a significant decrease (P<0.001) in IL8 was noted in the test versus the control group. The number of patients weaned off mechanical ventilation MV was significantly higher in the test versus the control groups (15 versus 6, P= 0.004, respectively). Survival and occurrence of side effects were comparable across groups. In conclusion**,** Administration of 10 g IV Vitamin C in 2 divided doses daily for 10 days in ARDS patients improved lung functions, pulmonary oxygenation, oxidative stress, and inflammatory markers. High-dose vitamin C reduced IL8 levels and facilitated weaning off MV. Vitamin C was tolerable with no significant side effects or drug interactions reported throughout the 10 days-treatment. (Clinicaltrials.gov Registration number: NCT03780933).

Keywords: *Vitamin C; ARDS; Nrf2; IL8; mechanical ventilation.*

1. INTRODUCTION

Acute Respiratory Distress Syndrome (ARDS) is a kind of acute hypoxemic respiratory failure characterized by excessive gas exchange and lung mechanical impairment, as well as a high case fatality rate **[1].** Various pharmacologic strategies including neuromuscular blocking agents, inhaled vasodilators, glucocorticoids, heparin, and aspirin have not been found to lower mortality in people with ARDS **[1-6]**. The priority in ARDS patient's care is determining and treating the underlying cause. Supportive therapy aims to avoid ventilator-associated lung

injury by combining lung-protective ventilation with conservative fluid management and nutrition to promote lung edema resorption and prevent the development of lung edema **[2, 6-9]**.

ARDS has a complicated etiology, with an imbalance of oxidant and antioxidant species, as well as inflammation. By altering lipids, proteins, and DNA, oxidative stress can cause cellular tissue damage and the production of secondary reactive species, which can lead to cell death via apoptosis **[10, 11]**. In an ARDS setting, there are plenty of possible sources of oxidative stress. including neutrophils, monocytes, macrophages, parenchymal cells, circulating oxidant-generating enzymes (xanthine oxidase), and high-oxygen inhaled gases, which are frequently used during (mechanical ventilation) MV **[12, 13]**. Endogenous antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase are expressed by cells to neutralize free radicals and counteract the negative effects of ROS. However, during an acute inflammatory response, these antioxidants are rapidly depleted **[10]**.

The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is an important regulator of antioxidant response element (ARE)-driven cytoprotective protein production. To avoid oxidative stress-induced cell and tissue damage, Nrf2 signaling must be activated **[14]**. In lung tissues, Nrf2 expression is relatively high, where detoxifying events take place at regular intervals and are triggered by several stimuli including oxidants, pro-oxidants, antioxidants, and chemopreventive agents. Against oxidative stress, aberrant inflammatory and immunological responses, apoptosis, and carcinogenesis, Nrf2 starts cellular rescue pathways. In the lungs, a lack of Nrf2 amplified the toxicity induced by a variety of oxidative assaults such as supplementary respiratory treatment, cigarette smoke, allergens, viruses, bacterial endotoxin,

and other inflammatory agents **[14]**. The role of Nrf2 stimulation in combating oxidative stress and inflammation in ARDS has been proven **[15]**. Previous research discovered that Nrf2 may be considered a possible gene of ARDS susceptibility in humans, with over 500 single-nucleotide polymorphisms (SNPs) of Nrf2 discovered, and the risk of ARDS has risen in people with a functioning NRF2 SNP in certain individuals **[16]**.

Furthermore, a multifaceted network of cytokines and other pro-inflammatory chemicals released by a range of cell types in the lungs initiates, amplifies, and modulates the inflammatory response in ARDS **[17]**. These inflammatory cytokines exacerbate inflammatory lung insult and endothelial permeability. High levels of the proinflammatory cytokines, tumor necrosis factor α, interleukins; 1 (IL-1), 6 (IL-6) and 8 (IL8) have been identified in the plasma of ARDS patients and were correlated with disease progression and patient outcome **[17, 18]**. In ARDS, the neutrophil attractant and activator interleukin 8 (IL-8) plays a key role. With an increased quantity of neutrophils in airspaces, neutrophils are thought to play a key role in the development and progression of ARDS **[19]**. Neutrophils have been found to contribute to lung tissue damage by facilitating microvascular damage. Increased numbers of alveolar neutrophils have been linked to poor survival and have been linked to both hypoxemia and increased lung permeability **[20]**.

Vitamin C, a powerful water-soluble antioxidant, regulates intracellular redox status by keeping sulfhydryl compounds, such as glutathione, in a reduced state **[21]**. Vitamin C is thought to be the most prevalent antioxidant in the extracellular fluid lining of the lung, and it contributes to the regeneration of membrane-bound oxidized vitamin E, allowing it to operate as a respiratory system chain-breaking

antioxidant. Vitamin C aids immune function by being transported into neutrophils and lymphocytes [21].

Generally, various pulmonary insults including ARDS have been linked to the production of reactive oxygen and nitrogenous species by pulmonary endothelial cells, leading to oxidative stress. Because the body's endogenous antioxidant capacity is overwhelmed by oxidative stress caused by these assaults, exogenous antioxidant supplementation is required and beneficial [22].

Unfortunately, the various antioxidants investigated did not show a significant influence on ARDS clinical outcome. Hence, the current research aimed to evaluate the impact of high dose Intravenous Vitamin C administration in ARDS patients, and assess its impact on the clinical outcome of ARDS patients.

2. PATIENTS AND METHODS

The present research was a prospective, randomized, controlled open-label study conducted on patients with moderate to severe ARDS admitted to the ICU of the National Center for Allergy and Chest Diseases, Cairo, Egypt. The research was carried out following the principles of the Helsinki Declaration. The Ethics Committees and institutional review boards of both the Faculty of Pharmacy, Ain Shams University, and the National Center for Allergy and Chest Diseases approved the study. The study was registered at Clinicaltrials.gov. Registration no. NCT03780933. All participants or their surrogates provided written informed consent. All ARDS patients admitted to the ICU were assessed for eligibility and were included if presented within 48 h of diagnosis with moderate to severe ARDS. Patients were excluded if they were; < 18 years, pregnant or lactating, allergic to vitamin C, with active kidney stones, not eligible to cardiopulmonary resuscitation (CPR), or

moribund, and not expected to survive 24 h.

Patients were categorized according to the Berlin classification of ARDS were divided into three severity categories: mild (200 millimeters of mercury (mm) Hg < $PaO_2/FiO_2 \leq 300$ mm Hg), moderate (100 mm Hg < $PaO_2/FiO_2 \leq 200$ mm Hg), severe ($PaO_2/FiO_2 \leq 100$ mm Hg) [18].

The current study is the first study to address the use of high dose IV vitamin C as an anti-inflammatory and antioxidant in critically ill ARDS patients, hence a priori power analysis could not be performed since there were no available studies to assist in the power calculation.

Eligible patients were randomly assigned using a simple randomization procedure with a (1:1) allocation ratio, to 1 of 2 treatment groups. Group 1 (Control); 20 ARDS patients received; conventional ICU management or Group 2 (Test); 20 ARDS patients received; conventional ICU management in addition to IV Vitamin C 10 g/day divided over 2 doses administered every 12 hours as an infusion, vitamin C was diluted in 250 mL NS and infused over 30 min daily for 10 days. The IV bag and the tubing were covered with aluminum foil to avoid Vitamin C photo-degradation. The study drug, Cevarol® 1 g IV ampoules were obtained from Memphis pharmaceutical company, Egypt.

The Vitamin C dose was adopted from previous studies in cancer patients [23-25].

All ARDS patients involved in the study received conventional ICU management and supportive treatment, including evidence-based treatment for ARDS including glucocorticoids, vasodilators, fluid support, and antibiotics in addition to low tidal volume ventilation and Positive end-expiratory pressure (PEEP).

Baseline assessment. All patients were assessed for the following; full history taking, clinical assessment, chest X-ray (CXR), High-

resolution CT chest, and laboratory assessment.

Laboratory assessment included; complete blood count (CBC), kidney and liver function tests, blood glucose (BG), arterial blood gases (ABGs), serum; Nrf2, interleukin 8 (IL8), and vitamin C levels.

NRf2, IL8, and Vitamin C levels were all measured in serum using SinoGeneClon kits and determined by ELISA technique using Tecan infinity F50 Microplate ELISA reader, Tecan; Germany. The kits were obtained from SinoGeneClon Biotech, USA.

For both groups, the clinical pharmacist assessed the occurrence and severity of side effects daily for 10 days.

Follow up. Both groups were followed up daily for the following: side effects occurrence, clinical outcome in terms of; PaO_2/FiO_2 ratio, need for extra ventilatory support, development of complications (sepsis, multi-organ dysfunction), and laboratory assessment. For the intervention group, serum levels of IL8, Nrf2, and Vitamin C were assessed on days 3, 5, and 7.

End of study assessment. The following was assessed for both groups; serum levels of Nrf2, IL8, and Vitamin C, PaO_2/FiO_2 ratio, occurrence and severity of side effects, the occurrence of complications, and weaning of mechanical ventilation.

2.1. Statistical methods

For data analysis and management, the Statistical Package for Social Sciences (SPSS) vs. 23 was used. The normality of numerical data was investigated using the Kolmogorov-Smirnov test and the Shapiro-Wilk test, and the results were summarized using medians and ranges, as appropriate. To summarize categorical data, numbers and percentages were used. Data exploration revealed that the obtained values are not normally distributed. The chi-square test was

used to make categorical data comparisons between the two groups. Within each period, the Mann-Whitney test was used to compare the two groups in terms of numerical variables. Friedman's test was used to assess change over time for each group. Following these two tests, the p-values were adjusted using post-hoc Bonferroni corrections. To determine the degree of correlation between the measurements, Spearman's correlation coefficients were determined. The overall survival time was calculated from the time of diagnosis until death or loss of follow-up. Survival rates were calculated using the Kaplan and Meier method. To determine the statistical significance of differences between survival curves, the log-rank test was used. The p-values are all two-sided. P-values of less than 0.05 were considered significant.

3. RESULTS

Out of a total of 98 ARDS patients presenting to the ICU and assessed for eligibility, only 75 patients matched the inclusion criteria and were randomly assigned to one of 2 arms (group control or test). Thirty-five patients were excluded during the study period and 40 patients continued the study, illustrated in the Consort flow diagram, **Fig. 1**.

At baseline, both groups were comparable with no significant difference between them regarding, demographics, serum levels of vitamin C, Nrf2, IL8, and lung functions (PaO_2/FiO_2), **Table 1**.

Vitamin C, Nrf2, and IL8 levels after treatment at 5 & 10 days are represented in **Table 2**.

Vitamin C levels significantly increased over the 5-10 days period in the test group, while it significantly decreased over time in the control group, **Fig. 2**.
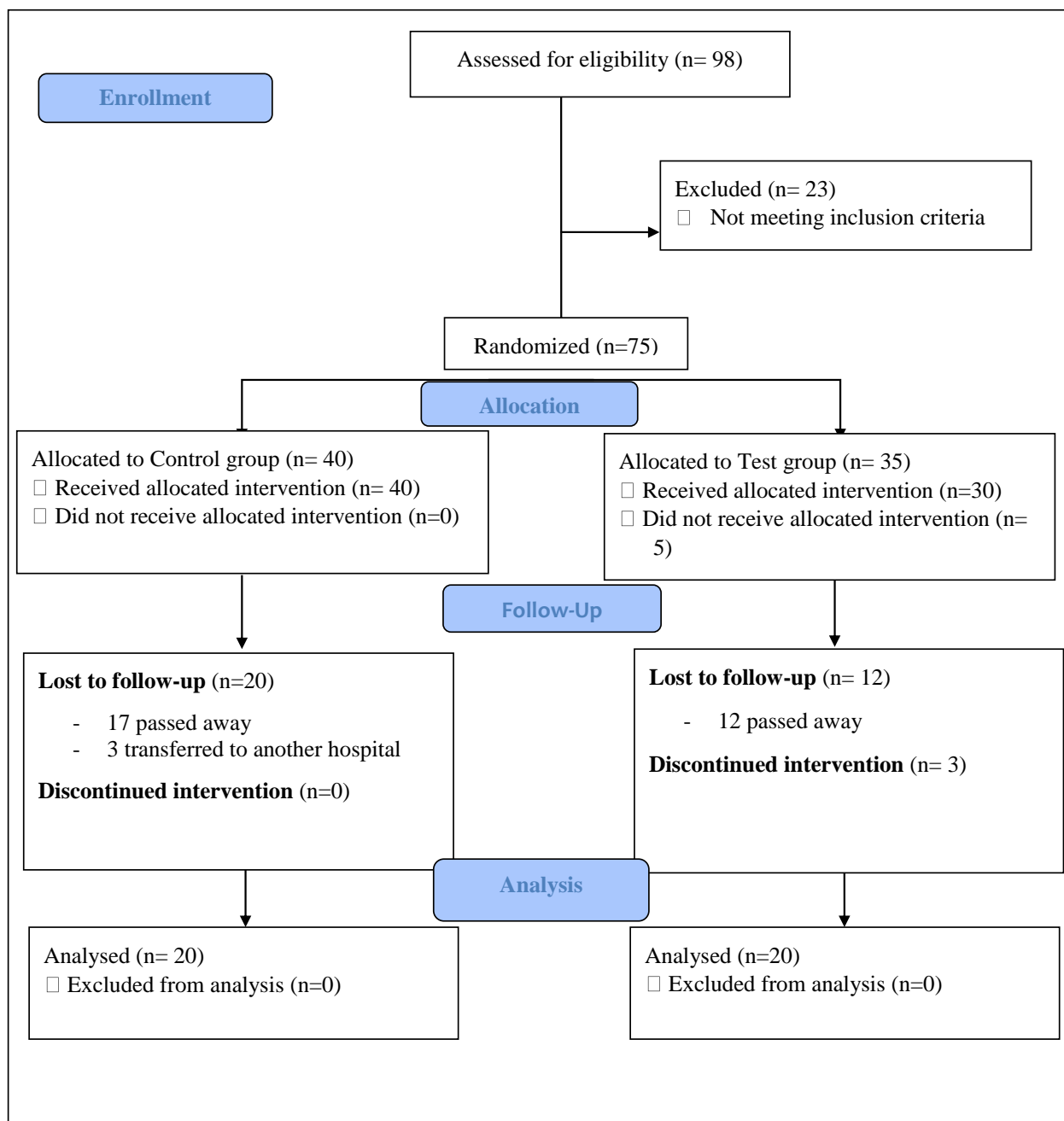
Assessed for eligibility (n= 98)

Enrollment

Excluded (n= 23)
- ☐ Not meeting inclusion criteria

Randomized (n=75)

Allocation

Allocated to Control group (n= 40)
☐ Received allocated intervention (n= 40)
☐ Did not receive allocated intervention (n=0)

Allocated to Test group (n= 35)
☐ Received allocated intervention (n=30)
☐ Did not receive allocated intervention (n= 5)

Follow-Up

Lost to follow-up (n=20)
- 17 passed away
- 3 transferred to another hospital

Discontinued intervention (n=0)

Lost to follow-up (n= 12)
- 12 passed away

Discontinued intervention (n= 3)

Analysis

Analysed (n= 20)
☐ Excluded from analysis (n=0)

Analysed (n=20)
☐ Excluded from analysis (n=0)

Fig. 1. Consort flow diagram

Table 1. Baseline patients Demographics, laboratory, and clinical data

Parameters	Control (n= 20)	Test (n= 20)	Significance P-value
Age (y); Mean±S.D	45.5 ± 12.3	41.5 ± 13.8	0.334[#]
Sex; n (%)			
Male	8 (40%)	8 (40%)	1.000[@]
Female	12 (60%)	12 (60%)	
Severity of ARDS;			
Moderate; n (%)	11 (55%)	14 (70%)	0.110[@]
Severe; n (%)	9 (45%)	6 (30%)	
Peripheral Blood Biomarkers; Median (IQR)			
Vitamin C (ng/mL)	384 (260-601)	401.1 (263.5-684.9)	0.660[$]
Nrf2 (pg/mL)	531.5 (413.0-801.3)	529.8 (270.3-937.7)	1.000[$]
IL8 (pg/mL)	323.1 (116.7-489.7)	186.1 (100.3-450.7)	0.020[$]
Lung Functions; median (IQR)			
PaO_2/FiO_2 on PEEP 5	101 (75-130)	84.5 (74-115)	0.072[$]

n; the number of patients, Nrf2; nuclear factor erythroid 2, IL8; interleukin 8, IQR: Interquartile range, PaO_2/FiO_2; the ratio of arterial oxygen partial pressure to fractional inspired oxygen, PEEP; positive end-expiratory pressure. Statistical test; $ Mann-Whitney test, # T-test, @ Chi-Square, $p \leq 0.05$ is considered significant.
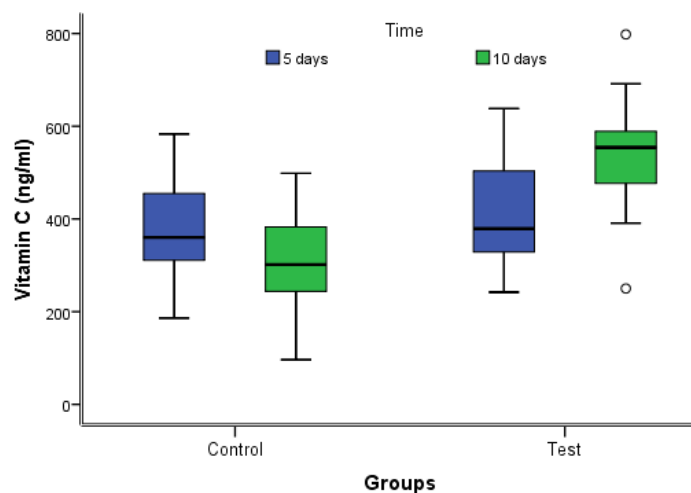
Fig. 2. Vitamin C levels in test and control groups after 5 and 10 days of treatment

5 days: Vitamin C levels after 5 days. 10 days: Vitamin C levels after 10 days. Control: ARDS patients who received conventional ICU management. Test: ARDS patients who received conventional ICU management in addition to 10g IV vitamin C. *Statistical test: Friedman's test, P< 0.001 (significant).

Table 2. Biomarkers & lung function assessment after 10 days treatment in the 2 groups

Parameter	Time (days)	Control (n= 20)	Test (n= 20)	P values Between groups [#]	time[†]
Vitamin C (ng/mL)	0	384 (260- 601)	401.1 (263.5- 684.9)	0.660	Control,
	5	360 (186-583)	379 (242- 638.4)	1.000	<0.001
	10	301.6 (96.3-498.6)	554.3 (250-798.1)	<0.00*	Test, 0.001
Vitamin C change 0-10; Median (IQR)		20.6 (8-63)	-24.6 (-134.9-37.2)	<0.00*	
Nrf2 (pg/mL)	0	531.5(413-801.3)	529.8 (270.3-937.7)	1.000	Control,
	5	446.2 (317.3-704.9)	450.8 (300.9-622.9)	1.000	<0.001
	10	333.3 (251.6-572.3)	592.6 (422.3-709.2)	<0.00*	Test <0.001
Nrf2 change 0-10; Median (IQR)		37.65(19.23-43.88)	-11.85(-93.97-39.75)	<0.00*	
IL8 (pg/mL)	0	323.1(116.7-489.7)	186.1 (100.3-450.7)	0.020	Control,
	5	423.1 (209.7-585.3)		<0.001	<0.001
			91.1 (45.5-167.1)		Test
	10	476.2 (255.9-688.2)	37.9 (11.4-109.6)	<0.001	<0.001
IL8 change 0-10; Median (IQR)		-58.19 (-181.66-6.62)	78.26 (60.25-94.8)	<0.00*	
Lung function assessment					
PaO$_2$/FiO$_2$ on PEEP 5	0	101 (75-130)	84.5 (74-115)	0.072	Control,1.0
	10	122.5 (100-200)	220 (135-350)	<0.00*	Test,<0.00*
PaO$_2$/FiO$_2$ on PEEP 5 change 0-10; Median (IQR)		-17.47 (-153.16-15.25)	-160.85 (-372.97-(-35))	<0.00*	

n; the number of patients, Nrf2; nuclear factor erythroid 2, IL8; interleukin 8, PaO$_2$/FiO$_2$; the ratio of arterial oxygen partial pressure to fractional inspired oxygen, PEEP; positive end-expiratory pressure. Statistical test; # Mann-Whitney test for comparisons between different groups; †Friedman's test for comparisons between different periods, p≤ 0.05 is considered significant.

Nrf2 levels significantly increased over the 5-10 days' time period in the test group, while the control group showed a significant decrease over time. The test group's Nrf2 levels were significantly higher than the control group at 10 days, **Fig. 3**.

IL8 levels significantly decreased over the 5-10 days' time period in the test group, while the control group showed a significant increase over time. The test group's IL8 levels were significantly lower versus the control group at 10 days, **Fig. 4**.

Regarding lung functions, PaO$_2$/FiO$_2$ showed a significant increase in the test group versus the control group at 10 days **Table 2**.

Using Spearman rho correlation, at baseline, the test group showed a high negative correlation between PaO2/FiO2 ratio and IL8 levels (r= -0.855, p<0.001). While, after 10 days of treatment, the control group showed a moderate positive correlation between the Vitamin C levels and PaO2/FiO2 ratio (r= 0.593, p= 0.006).

Weaning from mechanical ventilation and

mortality at the end of the study.

More patients were weaned from MV in the test group versus control (15 versus 6, p= 0.004, respectively). Mortality was comparable between the 2 groups (p= 0.327).
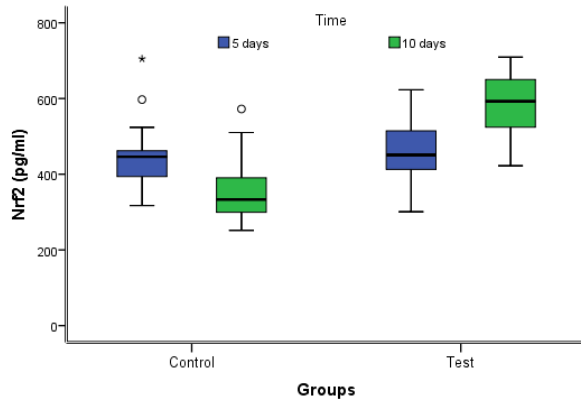

Fig. 3. Nrf2 levels in test and control groups after 5 and 10 days of treatment

5 days: Nrf2 levels after 5 days. 10 days: Nrf2 levels after 10 days. Control: ARDS patients who received conventional ICU management. Test: ARDS patients who received conventional ICU management in addition to 10g IV vitamin C. *Statistical test: Friedman's test, P< 0.001 (significant).

Fig. 4. IL8 levels in test and control groups after 5 and 10 days of treatment

5 days: IL8 levels after 5 days. 10 days: IL8 levels after 10 days. Control: ARDS patients who received conventional ICU management. Test: ARDS patients who received conventional ICU management in addition to 10g IV vitamin C. *Statistical test: Friedman's test, P< 0.001 (significant).
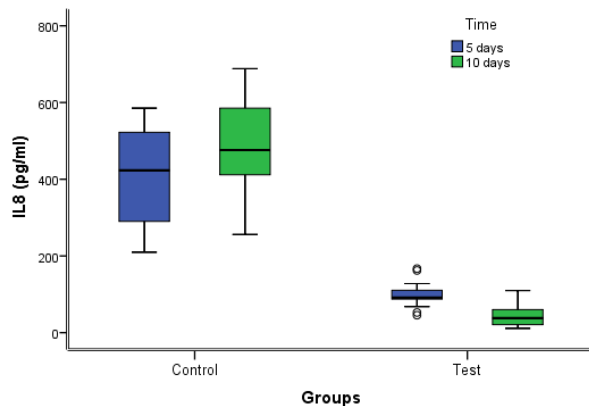
4. DISCUSSION

The recent research proves the first evidence for the effect of high dose Vitamin C administration on oxidative stress and inflammatory markers in critically ill patients with ARDS.

Both ventilatory and non-ventilatory strategies are used to treat ARDS. The most significant advances in lung injury patients' supportive care have been associated with improved ventilatory management strategies **[6, 26]**. Several clinical trials have been conducted to evaluate various pharmacologic options in ARDS, but none have demonstrated a reduction in either short-term or long-term mortality **[6, 7] [27, 28]**. The imbalance between oxidant and antioxidant species plays an important role in the pathophysiology of ARDS **[10]**. Antioxidant supplementation may delay or prevent the propagation of reactive oxygen and nitrogen species (ROS and RNS), respectively (RNS). The development of inflammatory disorders such as acute lung injury (ALI) and ARDS is linked to a disruption in the oxidant-antioxidant balance **[29]**. ARDS patients have shown a significant reduction in concentrations of reduced glutathione, ascorbic acid concentrations in the bronchoalveolar lavage fluid (BALF), α-tocopherol, β-carotene, and selenium versus healthy subjects **[30, 31]**.

Vitamin C levels in the plasma and leukocytes of critically ill patients are aberrant, and plasma ascorbate is inversely related to multiple organ failure but directly related to survival **[32, 33]**. Parenteral vitamin C, according to Fisher and colleagues, may play a role in the treatment of sepsis and ALI associated with sepsis **[34]**. Similarly, in the current study, the baseline Vitamin C levels were comparably low in both the test and control groups.

Nrf2, the transcription factor that is considered the "master regulator" of the antioxidant response [35], was significantly increased in the current study. After ten days of vitamin C supplementation, the test group showed a significant increase in Nrf2 levels, while the control group's levels declined. This was accompanied by a significant improvement in lung functions in the test versus control group, indicating the positive impact of vitamin C on modulating oxidative stress and improving pulmonary functions. To our knowledge, no research to date has looked into the role of high dose IV vitamin C administration on Nrf2 levels in patients with ARDS. In the lungs of patients with severe chronic obstructive pulmonary disease (COPD), Malhotra and colleagues found a decrease in the expression of NRF2-regulated antioxidant genes [36]. Moreover, the use of treatment modalities targeting the Nrf2 pathway in patients with COPD such as antioxidants halted COPD progression and prevented disease exacerbations [36].

Similarly, another study reported an up-regulation of genes coding for Nrf2 and several other important signaling molecules after IV vitamin C treatment. This activation of Nrf2, by IV vitamin C treatment, was proposed to protect against age-related degenerative diseases and acute insults to the lungs and cancer [37]. The possibility that IV vitamin C increases mRNA levels of NRf2 is of interest due to the link between inflammation and oxidative stress. Moreover, Mikivora and colleagues reported a significant increase in Nrf2 levels to post high dose IV vitamin C [37, 38].

The neutrophil attractant and activator IL-8 have been studied and found to play an important function in ALI/ARDS. IL-8 was found in significantly higher concentrations in ARDS patients' bronchoalveolar lavage fluids compared to controls, and ARDS patients with very high concentrations of IL-8 in bronchoalveolar lavage fluids had a higher mortality rate than patients with lower concentrations [20, 39, 40].

The current study showed high baseline levels of IL8 in the control and test group, indicating a high inflammatory status in ARDS patients. Similarly, several researchers have found that ARDS patients have greater serum IL-8 levels on day 1 than non-survivors and that these levels are considerably higher in non-survivors [41, 42]. The high link between plasma IL-8 concentrations and mortality was shown in a study by Ware and colleagues, indicating that plasma IL-8 plays the main role in the etiology of clinical ALI/ARDS [43].

Hirani *et al*. also found a link between IL-8 production and the progression of lung injury, because interleukin-8 is the most potent neutrophil chemokine in the lungs. In patients with established ARDS, it has been demonstrated to be responsible for more than half of the neutrophil chemotactic activity in BAL fluid [44].

After 10 days of vitamin C administration, there was a significant decline in IL8 levels in the test group versus their baseline levels and versus the control group, indicating the positive modulatory effect of vitamin C on the inflammatory burden in ARDS patients. The current study is the first study to demonstrate an impact on IL8 reduction in ARDS. Moreover, the current study demonstrated a significant improvement in PaO2/FiO2 in the test group versus the control group indicating the beneficial effects of vitamin C on pulmonary oxygenation. Similarly, a case report revealed the first use of IV vitamin C as a sepsis-induced ARDS treatment [45]. The current study also showed a highly negative significant correlation between IL8 levels and PaO2/FiO2 in the test group. These findings are in line with those of another research [45], which found that IL-8 levels were

considerably greater in patients who developed ARDS compared to those who did not and that high IL-8 levels were negatively associated with PaO2/FiO2.

In the current study, the test group had more patients weaned off mechanical ventilation versus control, indicating the positive impact of vitamin C administration on pulmonary oxygenation and hence the clinical outcome of ARDS patients. Following several studies that included Vitamin C as a component of the antioxidant strategy, vitamin C supplementation alone or in combination with other antioxidants was linked with a trend towards reduced mechanical ventilation time, length of intensive care unit (ICU) stay, and reduced multiorgan dysfunctional organs. These findings support the benefits of antioxidant-rich diets for ARDS patients **[46-48]**. Furthermore, a randomized, prospective clinical trial in critically ill surgical patients demonstrated that combining IV ascorbate (1,000 mg every 8 hours) and vitamin E reduced the relative risk of pulmonary morbidity and multiple organ failure, as well as shortened the time spent on the MV and ICU **[49]**. Similarly, another RCT found that severely burned patients who received high-dose ascorbate had lower morbidity **[50]**.

The current research did not report any remarkable side effects with vitamin C administration, except for GIT disturbances. Side effects were mild and did not require drug discontinuation or dose reduction. In accordance, there are no consistent or compelling data on serious health hazards of vitamin C administration in humans **[51]**. Finally, there was no significant occurrence of drug interactions in neither test or control groups.

Conclusion

Vitamin C 10 g IV added to conventional ICU management of ARDS was linked to a large increase in the antioxidant markers, Nrf2 levels, a decline in the inflammatory marker, IL8, and an increase in the number of patients weaned off mechanical ventilation. In terms of mortality, both groups were comparable.

Study limitations

The financial constraints associated with the cost of laboratory kits and diagnostic testing hampered additional patient recruitment. Also, patients' noncompliance with the research procedure resulted in multiple dropouts, necessitating re-enrollment and consuming additional time.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Research Ethical Committee, Faculty of Pharmacy, Ain Shams University, and conducted according to the regulations and recommendations of the Declaration of Helsinki. Written informed consents were signed and collected from all study participants.

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

Data analyzed during this study are all included in the main manuscript.

Competing interests

No competing interests were declared by the authors.

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