The effects of mechanical ventilation on oxidative stress

Os efeitos da ventilação mecânica no estresse oxidativo

INTRODUCTION

Invasive mechanical ventilation (IMV) consists of a positive pressure device connected to a tracheal tube. IMV is used for the treatment of patients with acute respiratory failure or in acute worsening of chronic respiratory failure. The purposes of IMV are to maintain gas exchange, to reduce respiratory muscle workload (increased in acute conditions with increased metabolic requirements), to reduce oxygen consumption, thereby reducing respiratory distress, and to allow for the use of specific therapies. (1)

Maintaining gas exchange with IMV has consequences to organ systems. Both higher positive pressures and inspired oxygen fractions greater than 21% are believed to cause oxidative stress levels so high that antioxidant methods are ineffective. (2)

The inspired oxygen fraction (FiO₂) is a mechanical ventilation parameter used to optimize tissue oxygenation. However, an
inappropriately adjusted FiO$_2$ can lead to the harmful effects of hypoxia or hyperoxia. Some tissue hypoxia consequences are cell changes and increased anaerobic metabolism. Toxic oxygen effects are not well established in humans; however, high or prolonged oxygen doses can cause both pulmonary and systemic injuries. In hyperoxia, most of the damage is caused by oxidative stress, which can cause organ molecule degeneration and consequent cell failure and death. Many intensive care unit (ICU) patients are on prolonged mechanical ventilation and therefore on prolonged oxygen use. These patients should receive FiO$_2$ levels sufficient to fit their metabolic requirements but not high enough to change their respiratory patterns and vital signs.$^{(3-5)}$

The severity and prognosis of ICU patients must be assessed.$^{(6)}$ The Acute Physiology and Chronic Health Evaluation II (APACHE II) is a useful and affordable prognostic score that was validated by Kruse and that is widely used in ICUs. This score is used for a range of diagnoses and is based on physiological parameters and clinical information that are available in most hospitals.$^{(7)}$

Growing evidence shows that oxidative stress has a relevant role in several clinical conditions, such as neoplasms, diabetes, atherosclerosis, neurovegetative diseases, chronic inflammatory diseases and ischemia/reperfusion injuries, among other common conditions in critically ill patients.$^{(8)}$

This article reports on a study aimed to assess and correlate oxidative stress in the plasma and erythrocytes of ICU patients undergoing IMV.

**METHODS**

This study was approved by the ethics committee of Faculdade NOVAFAPI - Teresina - Piauí, Brazil, and is considered to be in compliance with the Brazilian National Health Council’s Law 196/96 on research involving human subjects.

This was an observational, quantitative study assessing oxidative stress in patients admitted to the ICU of Hospital São Marcos, in Teresina, Piauí, Brazil.

Male and female patients between 18 and 80 years of age, admitted between September and October 2011 and signing a written informed consent form (ICF), were included during their first day on IMV. No exclusion criteria were applied.

**Data and sample collections**

After inclusion and completion of the subjects’ identification sheets, initial and final IMV respiratory monitoring data were collected. The data were collected daily, therefore allowing for the assessment until the day of mechanical ventilation discontinuation or death. The case report form included identification (name, age, weight, height, gender, APACHE II score, date of admission, underlying disease and comorbidities), date of each blood collection and ventilator parameters (ventilation mode, peak pressure [Ppeak], tidal volume [TV], inspiratory time [Ti], positive end-expiratory pressure [PEEP] and FiO$_2$), arterial blood gases (pH, PaO$_2$, PaCO$_2$, HCO$_3$), hemodynamic status (heart rate [HR] and mean blood pressure [MBP]) and peripheral oxygen saturation (SpO$_2$).

On the first and last days of IMV, 3-mL arterial blood samples were collected from the radial artery to analyze blood gases, and venous blood samples were collected for oxidative stress assessment. A GEM Premier 3000 blood gas meter was used for all blood gas measurements.

Oxidative stress was measured from 3-mL venous blood samples collected and stored in heparin and EDTA bottles. The blood was centrifuged to separate the plasma and erythrocytes. Plasma was stored and later analyzed for lipid peroxidation using the thiobarbituric acid-reacting substances (TBARS) technique. Erythrocytes were washed and centrifuged three times with saline 0.9%, discharging the supernatant. Following the last washing, erythrocyte precipitates were stored in acetic acid and magnesium sulfate solution for antioxidant enzyme analysis.

For lipid peroxidation and anti-oxidant enzyme assessment, the protein concentration in pulmonary tissue homogenate was calculated using the Bradford method, using bovine albumin as the standard. The samples were measured spectrophotometrically and the values expressed as mg/mL. Plasma lipid peroxidation was determined with the TBARS method. This technique consists of heating the material with thiobarbituric acid until the formation of a pink material, which is subsequently measured at 355 nm using a spectrophotometer. The results are expressed as nmol/mL TBARS.$^{(9)}$ Superoxide dismutase (SOD) anti-oxidant enzyme activity was measured on a plate reader, assessing epinephrine degradation and adenochrome formation; the data
are expressed as SOD units per mg of protein (U-SOD/mg prot.). Catalase (CAT) activity was measured with hydrogen peroxide in a plate reader at 240 nm, and the results are expressed as nmol/mg protein.

**Statistical analysis**

A data bank was prepared using Microsoft Excel software. The data were analyzed using the SPSS statistical package, version 17.0, and the results are expressed as the mean ± standard deviation. A significance level of 5% was adopted, with p < 0.05 considered statistically significant. Before/after comparisons were made using a pairwise t test, and correlations were tested with Pearson’s correlation test. The sample’s normality was assessed with the Kolmogorov-Smirnov test.

**RESULTS**

Table 1 displays the patients’ overall characteristics. Most of the patients were female — 75% of the sample. The mean age was 64.8 ± 17.6 years. The mean height was 155 ± 0.05 centimeters. The predicted weight was 47.7 ± 5.5 kilograms, and the predicted tidal volume (TV) was 382 ± 44.5 mL. The median APACHE II score was 15 ± 7.

<table>
<thead>
<tr>
<th>Table 1 - Anthropometric and clinical sample characteristics</th>
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</thead>
<tbody>
<tr>
<td><strong>Characteristics</strong></td>
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<tr>
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</tr>
<tr>
<td><strong>Gender</strong></td>
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<td><strong>Age (years)</strong></td>
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<td><strong>Height (m)</strong></td>
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<tr>
<td><strong>Predicted weight (Kg)</strong></td>
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<tr>
<td><strong>Predicted TV (mL)</strong></td>
</tr>
<tr>
<td><strong>APACHE II</strong></td>
</tr>
<tr>
<td><strong>IMV time (days)</strong></td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
</tr>
<tr>
<td>SH</td>
</tr>
<tr>
<td>DM</td>
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<tr>
<td>ARF</td>
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<tr>
<td><strong>Outcome</strong></td>
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<td>Death</td>
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<td>Discharge</td>
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TV - tidal volume; APACHE II - Acute Physiology and Chronic Health Disease scoring system II; SH - systemic hypertension; DM - diabetes mellitus; ARF - acute renal failure. The results are expressed as the mean ± standard deviation or as number (%).

The mean IMV time was 5.5 ± 3.5 days. The following comorbidities were recorded: three patients (25%) with systemic hypertension (SH), two patients (17%) with diabetes mellitus (DM) and two patients with acute renal failure (ARF) (17%). Six patients (50%) died during their hospitalizations.

Analyzing initial and final oxidative stress data, the TBARS levels were significantly higher after IMV (p < 0.05). No statistically significant differences were found for SOD or CAT; however, enzyme activity was identified as decreasing after IMV (Table 2).

No arterial blood gas parameters were shown to change significantly (Table 3). Similarly, as shown in table 4, the initial and final ventilation parameters were not different.

A statistically significant correlation was observed between FiO\textsubscript{2} and TBARS before (r = 0.4) and after (r = 0.4) IMV, suggesting that the higher FiO\textsubscript{2} was, the more oxidative stress there was (Figure 1).

Analyzing the correlation between the final PaO\textsubscript{2}/FiO\textsubscript{2} and final SOD, r = 0.51 (p < 0.05) was found, suggesting that patients with higher PaO\textsubscript{2}/FiO\textsubscript{2}...
Table 4 - Ventilation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial</th>
<th>Final</th>
<th>p value</th>
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<tr>
<td>Ppeak (mbar)</td>
<td>22.3 ± 3.3</td>
<td>21.5 ± 3.8</td>
<td>0.58</td>
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<tr>
<td>TV (mL)</td>
<td>517.2 ± 118</td>
<td>519 ± 196.1</td>
<td>0.97</td>
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<tr>
<td>Ti (s)</td>
<td>1.01 ± 0.05</td>
<td>1.03 ± 0.06</td>
<td>0.38</td>
</tr>
<tr>
<td>PEEP (mbar)</td>
<td>6 ± 1.5</td>
<td>6.75 ± 2.1</td>
<td>0.32</td>
</tr>
<tr>
<td>FiO₂</td>
<td>46.2 ± 10.2</td>
<td>38.7 ± 11.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Cest (mL/mbar)</td>
<td>41.5 ± 12.2</td>
<td>46.6 ± 19.4</td>
<td>0.44</td>
</tr>
<tr>
<td>R (mbar/mL/s)</td>
<td>13.9 ± 7</td>
<td>13 ± 3.5</td>
<td>0.69</td>
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The results are expressed as the mean ± standard deviation or percent. Ppeak - peak pressure; TV - tidal volume; Ti - inspiratory time; PEEP - positive end-expiratory pressure; FiO₂ - inspired oxygen fraction; Cest - static compliance; R - resistance.

Figure 1 - Analysis of the correlation between FiO₂ and thiobarbituric acid-reactive substances before and after invasive mechanical ventilation. A significant positive correlation was identified at the beginning (r = 0.4; p < 0.05) and end (r = 0.4; p < 0.05) of invasive mechanical ventilation. TBARS - thiobarbituric acid reactive substances.

Figure 2 - Correlation between PaO₂/FiO₂ and the antioxidant enzyme superoxide dismutase by the end of invasive mechanical ventilation. A significant positive correlation was observed (r = 0.51; p < 0.05). SOD - superoxide dismutase.

Figure 3 - Correlation between hospital length of stay and the antioxidant enzyme catalase by the end of invasive mechanical ventilation. A negative correlation was observed (r = -0.30; p < 0.05). CAT - catalase.
DISCUSSION

This study assessed the correlation between oxidative stress and IMV in ICU patients; our findings suggest that IMV might influence the redox state.

Analyzing oxidative stress after IMV, significantly increased TBARS levels were identified. Although the medical literature lacks reports from clinical trials assessing oxidative stress and IMV, our data agree with the findings of other authors who assessed biological models. Experimental animal and human oxidative stress studies have shown that increased metabolic activity favors oxidative molecular injuries. Oxidative stress, shown by increased TBARS, could be associated with endothelial dysfunction and prolonged IMV due to an increased production of oxygen-derived free radicals and also reactive oxygen species, such as hydrogen peroxide, causing both pulmonary and systemic injuries. Oxidative stress markers are remarkable tools for the assessment of possible effects on and implications for several metabolic processes, and they might be markers of pathologic and inflammatory processes. These substances can be measured in the blood, thus making them suitable for bedside care. Respiratory muscle contractions, either active or passive due to IMV, promote transient ischemia, while relaxation leads to reperfusion. This process characterizes the oxygen paradox in which a lack of oxygen followed by reperfusion leads to increased reactive oxygen species and can cause an unbalanced condition, characterizing oxidative stress.

Additionally, significant reductions of CAT and SOD anti-oxidant enzymes were observed after IMV. These serum enzyme decreases could be explained by the body’s reduced self-defense mechanisms, as the subjects were prolonged IMV patients with associated comorbidities.

The initial and final arterial blood gas and ventilation parameters demonstrated no statistically significant differences. However, the patients had increased oxidative stress and reduced levels of anti-oxidant enzymes. A significant correlation was observed between the initial FiO₂ and initial TBARS, as well as a correlation between the initial FiO₂ and final TBARS. This correlation shows that a higher FiO₂ leads to higher oxidative stress, as a high FiO₂, with the consequence of more circulating oxygen, causes oxidative injury. However, PaO₂ conditions that are too low can also cause oxidative stress.

Additionally, a correlation between the final PaO₂/FiO₂ ratio and final SOD was identified. Patients with higher PaO₂/FiO₂ ratios showed higher final SOD values, therefore showing that more oxygen from circulating FiO₂ might stimulate the production of SOD, increasing anti-oxidant defenses, as SOD acts by transforming superoxide radical anions (produced from oxygen) into hydrogen peroxide.

Analysis of the correlation between the IMV time and final CAT showed r = 0.3 (p < 0.05), demonstrating that patients with prolonged ventilation had higher CAT values by the end of IMV. This finding suggests that patients with less IMV time have more effective anti-oxidant systems and thus higher CAT concentrations. CAT’s anti-oxidant action takes place when free radicals reach the bloodstream via erythrocyte catalase. Catalase prevents meta-hemoglobin accumulation and also degrades hydrogen peroxide — a toxic metabolism byproduct — into water and molecular oxygen. Excessive H₂O₂ causes hemoglobin oxidation, consequently leading to a reduced oxygen concentration; this process can lead to infections, ulcers or even necrosis.

The predictive APACHE II score had a correlation with SOD anti-oxidant enzyme levels at the initial (r = 0.56; p < 0.05) and final assessments (r = 0.59; p < 0.05), suggesting that more severely ill patients had lower SOD values, both on the first and last days of IMV. This finding shows that, in general, ICU patients might have a higher oxidative stress burden as indicated by lower SOD levels.
oxidative parameters are associated with severity and outcome.\(^{24}\)

This study had some limitations: a) the small sample size; b) the lack of control patients, which would have allowed for the assessment of whether oxidative stress changes are secondary to underlying disease progression; and c) the use of TBARS as a single oxidative stress marker. Most analytes are generated during heating, which is part of the TBARS technique. Not adding butylated hydroxytoluene (BHT) can increase endogenous production of analytes, thus changing TBARS assessments.

Additional longitudinal studies and larger sample sizes are required to assess the predictive value of the selected variables, as well as the possibility of stratifying oxidative stress, anti-oxidant enzymes and patients’ outcomes.

**CONCLUSION**

This study’s results suggest that patients with invasive mechanical ventilation have increased oxidative stress by the end of IMV, with increased TBARS and reduced superoxide dismutase and catalase serum levels.

**REFERENCES**