The prevalence of diabetes mellitus (DM) has increased in recent years, principally due to the great number of patients with type 2 DM, which is related to the prevalence of obesity and sedentary lifestyle.\(^{(1)}\)

Functional abnormalities in the respiratory system, such as reduced lung elastic recoil, lung volumes and diffusing capacity, are caused by DM.\(^{(2-4)}\) Various cross-sectional studies\(^{(5-7)}\) have shown the effect that type 1 and 2 DM have on pulmonary function tests in adults. It is known that DM is an independent risk factor for the development of sleep apnea,\(^{(8)}\) and patients who present DM are more susceptible to contamination through airborne particulate matter.\(^{(9)}\)

One of the factors responsible for pulmonary alterations can be oxidative stress. The mechanism responsible for this development is hyperglycemia, which activates the polyol pathway, increasing the production of sorbitol. This increase results in cellular stress that leads to a decrease in the intracellular antioxidant defenses. It can also result in the concentration of the products of advanced glycosylation, thus altering cell function. However, hyperglycemia can also activate nuclear transcription factors, triggering an increase in the expression of the inflammatory mediators. The combination of these mechanisms alters the production of oxidants, causing cellular stress and consequently the structural damage.\(^{(10)}\)

With the objective of evaluating the increase of oxidative stress and possible damages to the lung structure caused by DM, the experimental
rat model of streptozotocin-induced DM was
developed. Histological techniques were used in
order to determine the alterations in the lung
structure; biochemical measurements were taken
in order to evaluate the oxidative injury, as were
blood gas measurements, in order to evaluate
gas exchange alterations.

This was a controlled experimental study
involving Wistar rats with a mean body weight
of 300 g. All animals were treated in accord-
ance with the World Health Organization
Ethical Code for Animal Experimentation. The
animals were divided into two groups, control
and diabetic, each comprising 10 animals.
The study period was 60 days, starting on the
day the diabetic animals presented glycemia
greater than 250 mg/dL. We induced DM using
an only intraperitoneal injection of streptozo-
tocin (70 mg/kg; Sigma Chemical, St. Louis, MO,
USA). A enzymatic colorimetric assay was used
to determine the glycemia.

On day 60 of the experiment, the animals
were sacrificed after having been i.p. anesthe-
tized with ketamine (100 mg/kg) and xylazine
(50 mg/kg). Subsequently, the thoraco-abdomi-
nal region was submitted to trichotomy, and a
mid-ventral laparotomy was conducted. Blood
from the abdominal aorta was collected in order
to evaluate the arterial blood gases. An ABL 700
analyzer (Radiometer, Copenhagen, Denmark)
was used to determine PaO\textsubscript{2}, PaCO\textsubscript{2}
and SaO\textsubscript{2}.
The lungs were removed and fixed in 4% para-
formaldehyde for histological analysis and stored
at -80ºC in order to subsequently quantify the
thiobarbituric acid reactive substances (TBARS)
and evaluate the activity of the antioxidant
carboxy dmutation (SOD).

In order to conduct the biochemical analysis,
the lung tissue was homogenized after which protein levels were quantified in accordance with the Lowry et al. method. Measurement of the TBARS was conducted as established by Buege & Aust. Determination of the SOD activity was performed according to the technique described by Misra & Fridovich.

The samples for the histological analysis
of the lung tissue were collected and stored
for 12 h in 10% formaldehyde solution, trans-
ferred to 70% alcohol and stained with H&E.
The anatomopathological examination was
performed in double-blind fashion by a patholo-

gist in the Pathology Laboratory of the Porto
Alegre Hospital de Clínicas.

Data were analyzed using the program
Statistical Package for the Social Sciences,
version 13 (SPSS Inc., Chicago, IL, USA). The
Student-Newman-Keuls test was used. In all
comparisons, the level of significance was set at
5%.

Blood glucose concentration was significantly
higher in the diabetic group when compared
with the control group, as in the evaluation of pulmonary lipid peroxidation, in which the
TBARS concentration was significantly higher
in the diabetic animals when compared with the
controls. When evaluating the antioxidant
enzyme SOD activity in the lung tissue, we
observed a significant decrease in the diabetic
group when compared with the controls.

In the blood gas analysis, we observed an
increased PaCO\textsubscript{2} in the diabetic group when
compared with the controls, and a decreased
PaO\textsubscript{2}. There was no difference regarding the
SaO\textsubscript{2} between the groups (Table 1).

In histology, we evidenced the presence of intravascular macrophages in the diabetic group, which suggests the presence of inflammatory
process. We also observed an increase in the extracellular matrix, expressed by the presence of fibrosis, as well as an increase in the thickness
of the alveolocapillary membrane (Figure 1).

In our study, we observed an increase in lung
oxidative stress in diabetic rats in relation to the
controls, as well as a decrease in the antioxidant
carboxy dmutation (SOD). Those data are in accord-
ance with the findings of other authors, who
demonstrated the increase of the oxidative stress

Table 1 - Comparison between the control group and the diabetic group in relation to the glycemia, lipid peroxidation, superoxide dismutase and blood gas analysis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Diabetic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS, nmol/mg protein</td>
<td>0.889 ± 0.17</td>
<td>1.585 ± 0.55*</td>
</tr>
<tr>
<td>SOD, IU/mg protein</td>
<td>14.35 ± 3.98**</td>
<td>4.64 ± 2.3</td>
</tr>
<tr>
<td>PaCO\textsubscript{2}, mmHg</td>
<td>46.2 ± 4.6</td>
<td>56.7 ± 9*</td>
</tr>
<tr>
<td>PaO\textsubscript{2}, mmHg</td>
<td>105.9 ± 9.3**</td>
<td>90.2 ± 17.1</td>
</tr>
<tr>
<td>SaO\textsubscript{2}, %</td>
<td>97.7 ± 0.4</td>
<td>95.7 ± 1.9</td>
</tr>
</tbody>
</table>

TBARS: thiobarbituric acid reactive substances; and SOD: superoxide dismutase enzyme. Values expressed as mean ± SD. *p < 0.05 vs. control group. **p < 0.05 vs. diabetic group.
and the decrease of the antioxidant enzyme SOD in the lungs of diabetic rats. Those authors also demonstrated that there is an increase in the expression of inducible nitric oxide synthase in the lung tissue of the diabetic animals. The same finding was exposed by another group of authors. However, they used, as an experimental model, alloxan-induced DM in rabbits. When the blood gas values were analyzed, we observed an alteration in the gas exchange in the diabetic animals, expressed through the decreased $\text{PaO}_2$ and the increased $\text{PaCO}_2$. This finding is described as an alteration frequently found in diabetic patients, who present a decreased diffusing capacity. This decrease can be correlated with the glycemic control and the duration of the disease. One of the factors responsible for this alteration can be the increase in the basement membrane thickness. In a study of the pulmonary biopsies of 171 patients, it was concluded that individuals with DM present an increase in basement membrane thickness similar to that seen in asthma patients. This increase was greater in individuals with cancer and pulmonary fibrosis, as well as in patients presenting TB or sarcoidosis.

When we analyzed the histology of the lung tissue, we found an increase in the alveolo-capillary barrier in the diabetic animals. These alterations were also observed by another group of authors, who studied the lungs of diabetic hamsters and concluded that these structural modifications sustain the functional disorders observed in the patients, and that the lung is one of the organs affected by DM. These alterations can be explained by variations in the synthesis of collagen and elastin, as well as by the fact that the phospholipid and phosphatidylcholine content is decreased in proportion to the alveolar surface. We also observe, in the lung tissue of diabetic animals, the occurrence of alterations in the morphology of the type II pneumocytes.

We concluded that oxidative stress is present in experimental DM, and that structural alterations in the pulmonary tissue are observed, as are alterations in blood gases.

References


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