EXPERIMENTAL STUDY

# The comparison of tracheostomy and translaryngeal intubation regarding free radical formation and pulmonary effects

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## ABSTRACT

**BACKGROUND:** Our aim in this study was to compare the blood gas changes, the malondialdehyde (MDA) and endogenous antioxidant glutathione (GSH) levels in blood and lung tissues after ischemia/reperfusion, the histopathological damage in lung tissue in rats provided respiratory support with mechanical ventilation after translaryngeal intubation and tracheostomy.

**METHODS:** Group 1 rats were provided mechanical ventilator support after translaryngeal intubation, Group 2 mechanical ventilator support after tracheostomy, and Group 3 was the control group where rats were only anesthetized. Three groups were compared for blood gas changes, MDA, GSH, and histopathological changes.

**RESULTS:** Blood gas evaluation showed a more marked increase in $pO_2$ values and decline in $pCO_2$ values in Group 2 than Group 1 ($p<0.05$), and higher serum MDA levels in Group 1 than Group 2 ($p<0.05$). Tissue GSH levels in Groups 1 and 2 were higher than the control group, but this difference was not statistically significant ($p>0.05$). In terms of histopathological scoring, the damage score in Group 1 was higher than in Group 2 ($p<0.05$).

**CONCLUSION:** This is the first study to show tracheostomy to be more advantageous than translaryngeal intubation in terms of blood gases, ischemia/reperfusion damage, and structural changes in the lung tissue.

**Keywords:** Ischemia/reperfusion injury; lung; tracheostomy; translaryngeal intubation.

## INTRODUCTION

Translaryngeal intubation and tracheostomy are standard and widely used procedures in patients who are planned to receive long-term mechanical ventilator support in intensive care units.[1,2] Long-term intubation has disadvantages and complications such as mouth, larynx, and trachea damage; malposition; parasinusitis; decreased patient comfort; and sedation requirement.[1–3] Tracheostomy is relatively more stable and provides an airway that can be tolerated better. It also has advantages such as leaving less dead space in the lungs, creating a short airway, and better lung oxygenation as

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a result of the easier cleaning of secretions.[1,3] Tracheostomy is therefore preferred over translaryngeal intubation in many intensive care units.[2,4]

The cells cannot preserve their integrity in case of hypoxia and ischemia, both of which may occur during ventilation treatment. Oxygenation of the cell at this stage causes the lipid acid radicals to react with oxygen and start lipid peroxidation. While malondialdehyde (MDA) is a product of lipid peroxidation that increases with the effect of free oxygen radicals, glutathione (GSH), and especially its reduced form, is a non-enzymatic endogenous antioxidant that protects the cell by interacting directly with these free oxygen radicals.[5–7] Lipid peroxidation causes tissue damage, leading to changes of MDA and GSH blood and tissue values. The histopathological evaluation of these tissues can also be used as indicator of tissue damage.[5,6,8,9]

The possible advantages of tracheostomy compared to translaryngeal intubation and the potential tracheal damage and complications have been investigated in previous clinical studies. However, there is no comparative experimental rat model study investigating the effect of these techniques on free oxygen radicals in the blood and lungs, and the structural changes they cause in the lung.[1,3,10–12]

The aim of this study was to compare blood gas changes, blood and tissue MDA and GSH values, and the histopathological damages that occur in the lung tissue in rats that are provided respiratory support with a mechanical ventilator after translaryngeal intubation or tracheostomy, on condition that the oxygen levels remain the same.

## MATERIALS AND METHODS

All animal studies were approved by the Experimental Ethics Committee. Wistar-Albino male adult rats (260–320 g) were kept in a room with a temperature of 24°C and 40% humidity using a 12 h light, 12 h darkness cycle.

Rats were divided into three groups of 15 each. Each rat was monitored in terms of temperature, pulse, and saturation changes every half hour with a rectal temperature probe and rat saturation monitoring device before ventilation. The control group rats in Group 3 were only administered anesthetic drugs and blood gas samples were taken afterward. Blood gases were studied and anticoagulated blood was drawn from the cardiac region before the sacrifice while the animal was under the effect of anesthesia. After cervical dislocation and sacrifice four hours later, rat lungs were dissected and tissue samples were taken from both lung lower lobes to evaluate the histopathology and tissue free radicals.

### Intubation and Tracheostomy Technique

After fasting overnight, rats were anesthetized with intramuscular ketamine hydrochloride (80 mg/kg, Ketalar; Eczacıbasi, İstanbul, Turkey) and 5 mg/kg Xylazine (Rhompun, Abdi İbrahim, Istanbul, Turkey).

The anesthetized rats were put in the supine position and the neck was put in extension. The trachea was entered by advancing a 14 G cannula over the tongue root accompanied by a guide. The guide was removed and the cannula was stabled in this region (Fig. 1).

After the vertical separation of the muscles by an incision at the midline, the cervical trachea was observed in the cervical region. An approximately 5-mm vertical incision was made on the anterior trachea wall and a 14 G cannula was passed through this incision. The rats were connected to the ventilator (Fig. 2).

### Ventilator Parameters

The rats in the first and the second group were connected to the SAR-830 Volume-Cycled respiration device and ventilated with a minimum ventilation flow rate of 250 ml/min.

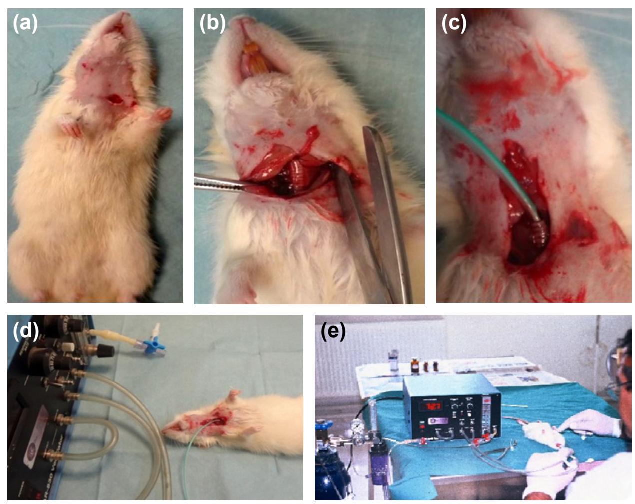


**Figure 1. (a-d)** Techniques of tracheostomy, **(e)** Techniques of intubation.

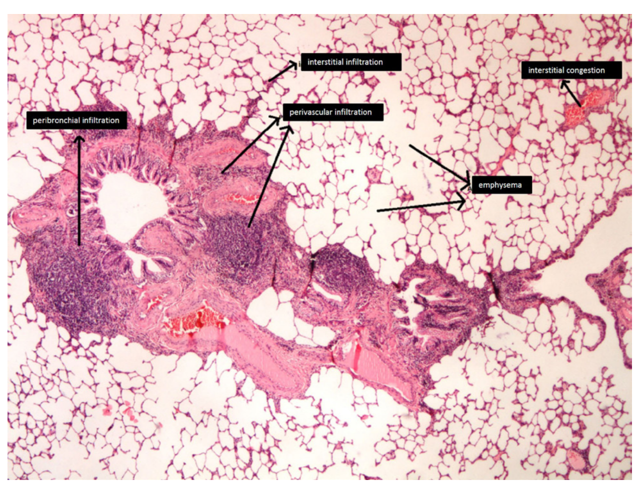


**Figure 2.** Histopathologic examination of lung sections – ×10 Hemotoxilen-Eosin.

The respiratory rate was set to 70–100/min and the rats were ventilated for a total of 4 h.

## The Evaluation of Biochemical and Metabolic Parameters

Blood gas samples were taken immediately after connection to the ventilator and also when disconnecting from the ventilator in all three groups. The blood pH, $pO_2$, $pCO_2$, and $HCO_3$ values were studied with the Easy Stat (Medica, Bedford, MA) blood gas analysis device. After the rats were disconnected from the ventilator, an anticoagulated 2 cc blood sample was obtained for plasma MDA and GSH level measurement. Tissue and plasma MDA levels were measured according to the method of Ohkawa et al.[8] Tissue MDA levels were expressed as nmol/g tissue. Tissue and plasma GSH levels were studied according to the method of Moron et al.[13] Tissue GSH levels were expressed as nmol/g tissue.

## Histopathological Evaluation

Two samples each were taken from both lung lower lobe peripherals of the sacrificed rats. The microscopic atelectasis, microscopic emphysema, perivascular edema, alveolar edema, congestion, alveolar hemorrhage, perivascular hemorrhage, alveolar mononuclear cell infiltration, interstitial edema and congestion, interstitial mononuclear cell infiltration, interstitial polymorphonuclear leukocyte (PNL) infiltration, peribronchial inflammatory cell infiltration, hyaline membrane formation, perivascular mononuclear infiltration, and pleural PNL infiltration parameters were evaluated semiquantitatively, with 0 indicating none, (≤5%) 1 indicating mild (>5–30%) 2, indicating diffuse (>30%) 3. Groups were scored according to the display of symptoms: absent 0, focal (≤5%) 1, (5–30%) 2, versus (>30%) 3. Scores were multiplied by each other and the total score was found 0–9.[14]

## Statistical Analysis

The data obtained from the experiments were analyzed with Kruskal–Wallis test and Mann–Whitney U test (SPSS 15.0; SPSS, Chicago, IL), $p<0.05$ was considered as significant.

# RESULTS

When the three groups were compared, the increase in $pO_2$ values and decrease in $pCO_2$ values after ventilation in Group 2 (tracheostomy) were found to be more marked than in Group 1 (translaryngeal intubation) and this difference was statistically significant ($p=0.066$ and $p=0.029$, respectively, $p<0.05$) (Table 1). The difference in pH values between the three groups was not statistically significant, but the values in the intubation group were lower than in the other two groups.

The serum MDA values in Group 1 were higher than in Group 2 and this difference was statistically significant ($p=0.001$, $p<0.05$). Tissue GSH values were higher in both the groups when compared to the control group, but this was not statistically significant ($p>0.05$) (Table 2).

There was a statistically significant difference between Group 1 and Group 2 in terms of the histopathological scores ([in-

**Table 1.** Mean blood gases analysis (pH, $pO_2$, $pCO_2$, $HCO_3$), temperature, pulse values, and standard deviations

| | Intubation (Group 1) | Tracheostomy (Group 2) | Control (Group 3) | p-value |
|---|---|---|---|---|
| pH | 7.27±0.03 | 7.36±0.01 | 7.37±0.10 | >0.05 |
| $pO_2$ | 40.6±6.09 | 58.33±8.25* | 50.96±5.62 | =0.066 |
| $pCO_2$ | 50.26±2.97 | 45.96±4.12* | 40.35±4.95 | =0.029 |
| $HCO_3$ | 23.44±1.56 | 28.48±1.13 | 20.62±2.03 | >0.05 |
| Temparature | 35.82±1.65 | 36.95±0.62 | 35.74±2.45 | >0.05 |
| Pulse | 108±3.24 | 112±2.84 | 115±5.6 | >0.05 |

*P<0.05.

**Table 2.** Serum and tissue mean MDA and GSH values and standard deviation

| | Intubation (Group 1) | Tracheostomy (Group 2) | Control (Group 3) | p-value |
|---|---|---|---|---|
| Serum MDA (nmol/ml) | 27.47±6.55* | 24.02±7.65* | 22.47±4.21 | =0.001 |
| Serum GSH (mg/dl) | 315.35±67.52 | 205.52±51.44 | 159.06±45.29 | >0.05 |
| Tissue MDA (nmol/g) | 145.43±45.32 | 205.4±36.45 | 116.29±31.48 | >0.05 |
| Tissue GSH (nmol/g) | 705.82±217.68* | 971.41±154.45* | 418.34±102.57 | =0.001 |

*P<0.05. MDA: Malondialdehyde; GSH: Glutathione.

**Table 3.** Histopathological scoring values showing lung damage

| | Intubation (Group 1) | Tracheostomy (Group 2) | Control (Group 3) | p-value |
|---|---|---|---|---|
| Score 0 | 2 | 7 | 8 | >0.05 |
| Score 1 | 7 | 7 | 6 | =0.002 |
| Score 2 | 6 | 1 | 1 | >0.05 |
| Mean score | 1.26±0.70 | 0.60±0.63 | 0.53±0.63 | =0.019* |

*P<0.05 was considered as significant.

tubation score 1.26±0.70, tracheostomy score 0.60±0.63] [p=0.019, p<0.05]). After the assessment of the total scores, in 1 and 2, representing damage, were more common in the intubation group (p=0.002). There was no statistically significant difference between the groups as regards microscopic atelectasis, emphysema, alveolar edema, alveolar hemorrhage, hyaline membrane formation, alveolar mononuclear leukocyte (MNL) infiltration, interstitial edema and congestion, interstitial MNL infiltration, and inflammatory peribronchial mononuclear infiltration. A statistically significant difference was present between the two groups in terms of pleural neutrophilic infiltration (p=0.0001) and perivascular mononuclear infiltration (p=0.001) (Table 3).

## DISCUSSION

Tracheostomy is preferred to translaryngeal intubation in many intensive care units for patients scheduled to receive long-term support with a mechanical ventilator as it provides a more effective airway, reduces dead space more effectively, provides a short airway, and oxygenates lungs better with secretions cleaned more easily.[1,2] There are many previous studies on the possible advantages of tracheostomy compared to translaryngeal intubation and the resulting tracheal damage and complications, but there is no study on an experimental model showing blood oxygen levels as well as microscopic and biochemical pulmonary changes, as in our study.[3,10–12,14]

Oxygen pressure changes have complex effects on cardiovascular and pulmonary function in the body. These effects lead to various reactions in many cell components such as the cell membrane, enzymes, and ion channels, and also to changes in cell functions.[15] In addition, hyperoxia and hypoxia stimulate ventilation and decrease arterial carbon dioxide pressure, leading to oxidative stress in the body and increasing reactive free oxygen radical formation.[16–18] The brain and lung are the most sensitive organs to these oxygen-related changes.[7] Rats were used in our study as such respiratory problems in rats cause changes similar to those in other mammals and adaptation mechanisms are quite similar to those in humans.[16–19]

The lung can be exposed to hypoxia during ventilation treatment both in patients who have undergone translaryngeal intubation or tracheostomy. This situation causes changes in the blood gas parameters of the patients.[5] We found in our study that the rats in the intubation group were more exposed to hypoxic conditions than the rats in the tracheostomy group after ventilation therapy and the decrease in oxygen pressure values and increase in $CO_2$ were statistically significantly more pronounced. No difference was present between the groups in terms of the metabolic parameters of temperature and pulse changes. There was also no statistically significant difference between the three groups in terms of blood pH values, but the values of the rats in the intubation group were closer to acidosis.

Cells cannot preserve their integrity during ischemia. If oxygenation in the cell occurs at this stage, the lipid acid radicals react with oxygen and start lipid peroxidation. This condition can be measured not only in the blood but also in the tissues.[5,6,8]

MDA is a product of lipid peroxidation that increases with the effect of free oxygen radicals. Its presence in the blood in large amounts indicates increased lipid peroxidation. Lipid peroxidation increases membrane permeability and stimulates the chemotaxis of leukocytes to the region.[6] If translaryngeal intubation provides less oxygenation compared to tracheostomy in patients treated with mechanical ventilation, free oxygen radicals and lipid peroxidation destruction products can be expected to be higher in the blood and tissues of these patients, as shown in other studies. The fact that serum MDA levels were higher in the rats in the intubation group compared to the tracheostomy group in our study supports the appearance of a larger amount of free oxygen radicals in the intubation group. GSH is a tripeptide that is synthesized from glutamate, cysteine, and glycine and is one of the most important intracellular antioxidant molecules. The reduced form is reported to especially protect the cell against free radicals by directly interacting with radicals such as hydrogen peroxides, hydroxyl, superoxide, and alkoxyl.[20] We believe that the reason for the higher tissue GSH in both study groups compared to the control group in this study is related to the volume trauma effect due to the ventilator settings.

Damages such as pulmonary edema, atelectasis, consolidation, fibrin formation, congestion, inflammation, arteriolar thickening, and hyalinization as well as hypertrophy and hyperplasia of alveolar cells may occur due to the partial pressure of the inspired oxygen. The lung edema and increased permeability due to the effect of high-pressure oxygen are reported to be associated with the increase of reactive oxygen radicals.[21] The damage in the lungs of rats in the tracheostomy group was observed to be lower than in the intubation group according to the total histopathological change scores. The increase in perivascular mononuclear cell and pleural PNL infiltration that are indirect damage indicators was observed to be statistically significantly higher in the intubation group compared to the tracheostomy group.

## Conclusion

We have shown microscopically and biochemically that the pulmonary damage in rats that have undergone intubation is more extensive than in rats with tracheostomy only and that values of MDA, the last product of lipid peroxidation, and GSH, an indicator of the antioxidant defense mechanism, are higher in the lung tissue and blood. Biochemical changes and tissue damages were presented first time in this rat study.

We would like to thank Beyza Akdağ who made the statistical calculations of the work.

**Ethics Committee Approval:** This study was approved by the Pamukkale University Animal Experimental Ethics Committee (Date: 17.11.2019, Decision No: B.30.2.PAÜ.0.01.00.00.400-1/44).

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**Authorship Contributions:** Concept: F.T., C.A.; Design: G.Y.; Supervision: F.T., Y.E.; Resource: B.Ş.; Materials: H.A., F.B., B.Ş., H.At.; Data: F.T., G.Y.; Analysis: C.A.Ö.; Literature search: Y.E.; Writing: F.T.; Critical revision: C.A.Ö., F.B., H.A.

**Conflict of Interest:** None declared.

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DENEYSEL ÇALIŞMA - *ÖZET*

# Trakeostomi ve translaringeal entübasyonun serbest oksijen radikalleri ve akciğer dokusu üzerine etkisi

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AMAÇ: Translarengeal entübasyon ve trakeostomi uygulandıktan sonra mekanik ventilatörle solunum desteği sağlanan sıçanlarda, oluşan kan gazı değişiklikleri, iskemi/reperfüzyon sonucu ortaya çıkan malondialdehit ve endojen antioksidan glutatyonun kan ve akciğer dokusundaki değerleri ile akciğer dokusunda meydana gelmiş histopatolojik hasarlanmaların karşılaştırılmasını amaçladık.

GEREÇ VE YÖNTEM: Grup1; translarengeal entübasyon, grup 2; trakeostomi uygulandıktan sonra mekanik ventilatör desteği sağlanan, grup 3; ise yalnızca anestezi uygulanmış kontrol grubu sıçanlardan oluşmaktaydı. Meydana gelen kan gazı değişiklikleri, MDA ve GSH düzeyleri ile akciğer dokusunda oluşmuş histopatolojik değişikliklerin gruplararası karşılaştırılmaları yapıldı.

BULGULAR: Grup 2'de, $pO_2$ değerlerindeki artış ve $pCO_2$ değerlerinde ki düşüşün grup 1'e göre daha yüksek olduğu ($p<0.05$), grup 1'deki serum MDA değerlerinin grup 2'ye göre daha yüksek olduğu saptandı ($p<0.05$). Doku GSH değerleri ise her iki grupta da kontrol grubuna göre sayısal olarak yüksekti, ancak bu fark istatistiksel olarak anlamlı değildi ($p>0.05$). Histopatolojik incelemeler sonucu yapılan skorlama açısından ise grup 1'deki hasar skorunun grup 2'deki hasar skoruna göre daha fazla olduğu saptandı ($p<0.05$).

TARTIŞMA: Kan gazı değişiklikleri ve ortaya çıkan iskemi/reperfüzyon hasarlanmasının serbest oksijen radikallere olan etkisi ile akciğer dokusunda oluşturduğu yapısal değişiklikler açısından trakeostominin translarengeal entübasyona göre daha avantajlı olduğu ilk kez bu deneysel çalışmayla ortaya konmuştur.

Anahtar sözcükler: Akciğer; iskemi/reperfüzyon hasarı; trakeostomi; translarenjial entübasyon.

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