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Original Article

Investigation of Oxidative Stress Parameters and Prolidase Enzyme Level Change in Mechanically Ventilated Patients in Intensive Care Unit

Yoğun Bakımda Mekanik Ventilasyon Uygulanan Hastalarda Oksidatif Stres Parametreleri ile Prolidaz Enzim Düzeyi Değişiminin Araştırılması

Erdoğan Duran [1*], Zeynep Baysal [1], Şaban Yalçın [1], Harun Aydoğan[1], Hakim Çelik[2], Mahmut Alp Karahan[1], Nurten Aksoy[2]

[1]Harran University Faculty of Medicine Hospital, Department of Anesthesiology and Reanimation Sanliurfa, Turkey
[2]Harran University Faculty of Medicine Hospital, Department of Biochemistry Sanliurfa, Turkey

***Corresponding author:**

Dr. Erdoğan DURAN

Adress: Harran University Faculty of Medicine Hospital, Department of Anesthesiology and Reanimation 63000 Sanliurfa, Turkey

email: drerdoganduran6331@gmail.com

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Abstract

Background: Mechanical ventilation (MV) therapy can increase oxidative stress in the lungs. Oxidative stress can cause damage to collagen, an important component of the extracellular matrix in the lungs. In this study, total oxidative (TOS) and antioxidative (TAS) status in serum and bronchoalveolar lavage (BAL) were determined in patients who underwent MV. Our aim in the study was to determine whether there is a relationship between MV duration and oxidative stress parameters and prolidase, a collagen turn-over marker, in serum and BAL. **Materials and Methods:** The study included 26 adult patients aged 30-70 years, without primary lung pathology, and who were treated with MV for respiratory failure. TOS-TAS and prolidase enzyme activity were studied to determine oxidative stress levels in blood and BAL samples taken from patients on days 1-3-5. The oxidative stress index (OSI) was found by calculating the TOS/TAS ratio.

Results: As the MV treatment period of the patients gets longer, in the serum; It was observed that OSI and prolidase levels increased significantly ($p<0.01$). Also in BAL; It was determined that the TAS decreased significantly, the OSI increased significantly and the prolidase level decreased significantly ($p<0.001$).

Conclusion: Depending on the duration of MV therapy, increased oxidants (and decreased antioxidant capacity) in serum and BAL cause collagen tissue damage in the lungs. Prolidase in serum has also increased as an indicator of collagen damage in the lungs. We think that serum prolidase activity can be used as a viable test in order to detect and control MV-induced lung injury at an earlier stage.

Key Words: Mechanical Ventilation, Lung Damage, Reactive Oxygen Derivatives, Oxidative Stress, Prolidase

Öz

Amaç: Mekanik ventilasyon (MV) tedavisi akciğerlerde oksidatif stresi arttırabilir. Oksidatif stres, akciğerlerde ekstraselüler matriksin önemli komponenti olan kollajenin hasarına neden olabilir. Bu çalışmada, MV uygulanan hastalarda, serum ve bronkoalveolar lavajda (BAL) total oksidatif (TOS) ve antioksidatif (TAS) durum belirlendi. Çalışmadaki amacımız, serumda ve BAL'da, MV süresi ile oksidatif stres parametreleri ve bir kollajen turn-over markeri olan prolidaz arasında bir ilişki olup olmadığını tespit edebilmekti. **Gereç ve Yöntem:** Çalışmaya, yaş aralığı 30–70 olan, primer akciğer patolojisi olmayan, solunum yetmezliği tanısıyla MV tedavisi gören, 26 erişkin hasta dahil edildi. Hastalardan 1- 3- 5- 7. günlerde alınan kan ve BAL örneklerinde, oksidatif stres seviyelerinin belirlenmesi için TOS-TAS ve prolidaz enzim aktivitesi çalışıldı. TOS/TAS oranı hesaplanarak, oksidatif stres indeksi (OSİ) bulundu. **Bulgular:** Hastaların yoğun bakımda kalış süreleri ve MV süresi uzadıkça serumda; TAS'ın anlamlı olarak ($p<0,001$) azaldığı, TOS ve OSİ'nin anlamlı şekilde ($p<0,01$) arttığı, prolidaz seviyesin anlamlı olarak ($p<0,001$) arttığı görüldü. BAL'da; TAS'ın anlamlı olarak ($p<0,001$) azaldığı, TOS ve OSİ'nin anlamlı olarak ($p<0,001$) arttığı, prolidaz seviyesin anlamlı olarak ($p<0,001$) azaldığı tespit edildi.
Sonuç; MV tedavisi süresine bağlı olarak, serum ve BAL'da artan oksidanlar (ve azalan antioksidan kapasite) akciğerlerde kollajen doku hasarına neden olmaktadır. Akciğerlerdeki kollajen hasarının göstergesi olarak serumdaki prolidaz da artmaktadır. MV'a bağlı akciğer hasarının, daha erken dönemde belirlenip kontrol altına alınabilmesi açısından, uygulanabilir bir test olarak serum prolidaz enzim aktivitesinden faydalanılabileceği düşüncesindeyiz. Çalışmamızın ileri klinik çalışmalarla desteklenmesi uygun olacaktır.

Anahtar Kelimeler: Mekanik Ventilasyon, Akciğer Hasarı, Reaktif Oksijen Türevleri, Oksidatif Stres, Prolidaz.

Introduction

Mechanical ventilation (MV) therapy, which is inevitable to be applied when necessary, can cause barotrauma and oxygen toxicity in the lungs in correlation with the current state of the patient's lungs and the length of stay in the ventilator (1).

Free radicals can occur during the course of normal metabolic events, as well as the metabolism of some foreign substances, exposure of the organism to external factors such as radiation. It is known that free radicals play a role in the pathophysiology of acute inflammatory conditions, severe sepsis, acute lung injury, emphysema, bronchopulmonary dysplasia, pneumoconiosis, adult respiratory distress syndrome, and multi-organ failure (2).

Prolidase is a specific enzyme, which is involved in the metabolic cycle of collagen, which is in the structure of many tissues in the body, and takes an active role in the destruction of collagen and re-participation of proline in the collagen production cycle. Since collagen is a structural component of many organs and tissues, as well as the extracellular matrix, it is affected by the pathology of these structures (3-6).

We also in this study, we aimed to investigate whether changes in oxidative stress parameters and prolidase enzyme levels in serum and bronchoalveolar lavage (BAL) could be used as an early-stage marker of lung injury in intensive care patients undergoing MV.

Material and Method

Local ethics committee approval (12 February 2009, session 02) and informed consent of patients' relatives were obtained before the study. The study was carried out in the General Intensive Care Unit of Harran University Faculty of Medicine (Department of Anesthesiology and Reanimation). The study included 26 adult patients aged 30-70 years who were treated with MV in the intensive care unit and had no primary lung pathology. Demographic data of the patients, hemogram-biochemistry-blood gas values and coma scores on the 1st, 3rd, 5th and 7th days were recorded.

The mechanical ventilator mode was set to Synchronized Intermittent Mandatory Ventilation (SIMV). Fraction of Inspired Oxygen (FiO2) was started at 100% when the patient was connected to the mechanical ventilator, gradually decreasing according to the blood gas results and fixed to 40% as soon as possible (within the first three hours at the latest). The tidal volume was standardized as 6 milliliter (ml)/kg, positive end expiratory pressure (PEEP) 5 cmH2O, respiratory rate 10-12/min, Inspiration/Expiration 1:2. Ventilator settings were adjusted in the morning and evening, targeting the values blood gas results (pH: 7.35 – 7.45, PCO2: 35-45 mmHg, PO2: 80-100 mmHg, HCO3: 22-26 mmol/liter [L]).

Enteral nutrition support was started and fluid therapy was arranged for the patients. Hemoglobin level was tried to be kept between 8-12 grams (g)/dl and replacement was done when necessary. BAL samples and blood samples were collected by non-bronchoscopic telescopic method from patients who were connected to mechanical ventilators on days 1-3-5-7. BAL samples were obtained with the help of a non-bronchoscopic telescopic BAL catheter (Combicath, Plastimed, France) after 20 mL of sterile saline was given through the endotracheal tube. The obtained samples were centrifuged at 3000 rpm for 5 minutes and stored in a deep freezer at -80ºC until the working time. Hemodynamic and ventilator parameters and arterial blood gas values of the patients were recorded. Prolidase enzyme activity, Total Antioxidant Level (TAS) and Total Oxidant Level (TOS) were measured in serum and BAL samples. The optimized modified Chinard Method (5), which is a colorimetric measurement method for prolidase enzyme activity, and a fully automatic colorimetric method developed by Erel (Rel-Assay commercial kits) were used to measure total antioxidant level and total oxidant level (6). Oxidative Stress Index (OSI); It was calculated by dividing TOS by TAS.

Statistical Assessment: Statistical Package for Social Sciences (SPSS) for Windows 25.0 (SPSS Inc. Chicago USA) program was used for statistical analysis. Repeated Analysis of Variance was used for multiple comparisons between groups and Bonferroni Test was used for within-group comparisons. Results were expressed as mean±standard deviation, and $p<0.05$ was considered statistically significant.

Results

When the demographic data of the patients were examined; 19 (73.1%) of the patients were male, 7 (26.9%) were female, mean age 64.3±14.13, mean weight 74.46 kg±14.07 kg, mean height was recorded as 167.69 cm±6.94 cm. Patients' APACHE II values were 23.7±4.6 on day 1; 3rd day 23.5±4.8; 5th day 24.7±5.3; Day 7 was 23.7±6.7, SOFA values were 6.7±2.7 on day 1; 3.day 6.9±2.7; 5th day 7.7±3.2; It was determined as 7.9±3.1 on the 7th day.When the serum TAS values between the 1st, 3rd, 5th and 7th days of the patients were compared within themselves, a statistically significant difference was found. It was determined that TAS values decreased significantly as the length of stay in the intensive care unit of the patients ($p<0.001$) (Table 1).

When the serum TOS values of the patients were compared, the TOS values were found to be statistically significantly higher on the 3rd day compared to the 1st day ($p<0.01$). A statistically insignificant increase ($p>0.05$) was observed on the fifth day compared to the first day, and a statistically insignificant decrease was observed compared to the third day ($p>0.05$). The serum TOS values of the patients were found to be significantly increased on the 7th day (compared to the 1-3-5 days) ($p<0.01$) (Table 1).

The serum OSI values of the patients, such as TOS values, were found to be statistically significantly higher on the 3rd day compared to the 1st day ($p<0.01$). A statistically insignificant increase ($p>0.05$) was observed on the fifth day compared to the first day, and a statistically insignificant decrease was observed compared to the third day ($p>0.05$). The serum OSI values of the patients were found to be significantly increased on the 7th day (compared to the 1-3-5 days) ($p<0.01$) (Table 1).

Serum prolidase activities were found to be statistically significantly increased on day 3 compared to day 1 ($p<0.01$). On the fifth day, prolidase levels were found to be statistically significant ($p<0.01$) compared to the 1st day and insignificantly higher than the 3rd day ($p> 0.05$). The increase in serum prolidase levels on the seventh day was not statistically significant compared to the 5th day ($p>0.05$), and it was found to be statistically significant compared to the 1st and 3rd days ($p<0.001$) (Table 1)(Figure 1).

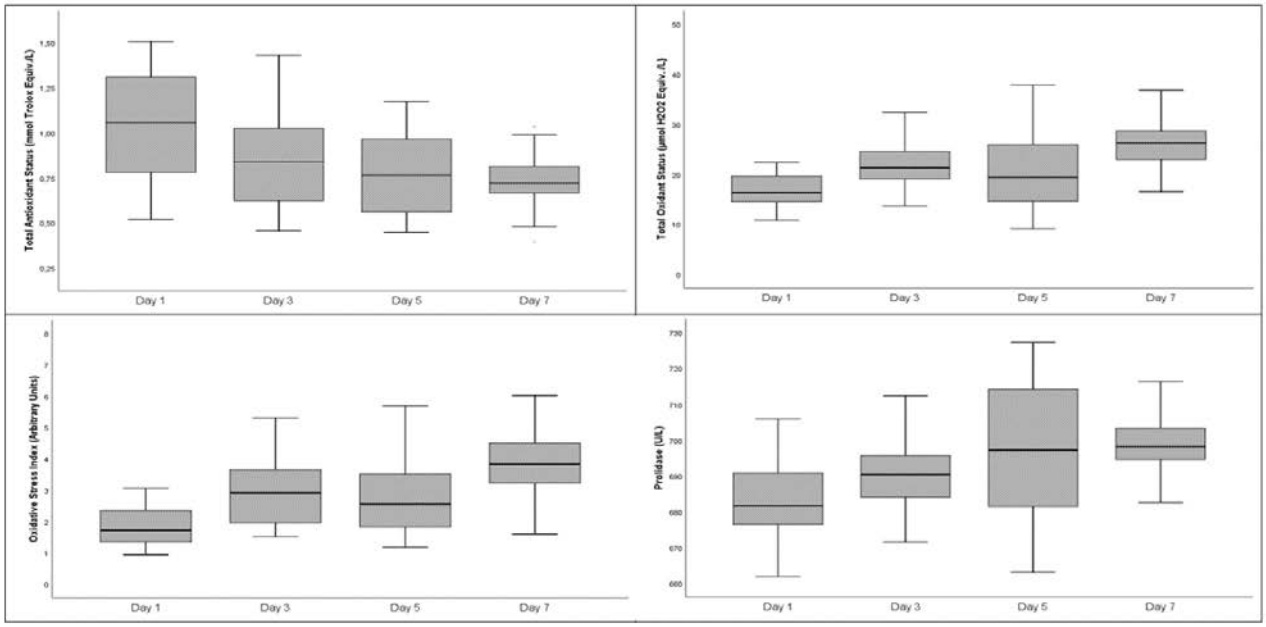
Table 1. Serum prolidase and serum Oxidant/Antioxidant parameters of the patients

	Serum Day1 (n= 26)	**Serum Day 3 (n= 26)**	**Serum Day5 (n= 26)**	**Serum Day 7 (n= 26)**	**P**
Prolidase (U/L)	682.31±11.73	691.30±12.63^{a**}	696.20±18.73^{b**}	699.95±10.48c***,e**	<0.001
Total Oxidant Level (µmol H2O2 Eqv./L)	17.26±3.87	22.85±6.45^{a**}	20.53±7.29	26.91±7.01^{c***,e**,f***}	<0.001
Total Antioxidant Level (mmol Troloks Eqv./L)	1.02±0.31	0.85±0.26^{a**}	0.76±0.21^{b***,d**}	0.72±0.16^{c***,e**}	<0.001
Oxidative Stress Index (AU)	1.81±0.60	2.89±1.06^{a***}	2.96±1.50^{b**}	3.87±1.22^{c***,e***,f***}	<0.001

a: 1 to 3; b: 1 to 5; c: 1 to 7; d: 3 to 5; e: 3 to 7; f: There is a significant difference between the 5th and 7th day. ***: p≤0.001; **: p≤0.01; *: p≤0.05

When the BAL TAS values of the patients were compared, it was determined that the BAL TAS values decreased statistically significantly ($p<0.05$) on the 3rd day compared to the 1st day. BAL TAS values; on the 5th day; It was found to be statistically significantly higher than the 3rd day ($p <0.01$), and statistically insignificantly higher than the 1st day ($p>0.05$). On the 7th day; It was found significantly ($p<0.01$) and ($p<0.001$) lower than the 1st and 5th days, and insignificantly ($p>0.05$) lower than the 3rd day (Table 2)

Figure 1. Serum of the patients on the 1st. 3rd. 5th and 7th days; Difference. distribution and standard deviations between TAS. TOS. OSI. Prolidases



When the BAL TOS values of the patients were compared, we found that the values decreased statistically significantly on the 3rd day compared to the 1st day ($p<0.001$). BAL TOS values were found to be statistically significantly higher on the 5th day compared to the 1st and 3rd days ($p<0.01$), ($p<0.001$). BAL TOS values were found to be increased statistically significantly ($p<0.001$) on the 7th day compared to the 1st and 3rd days, but in a statistically insignificant way ($p>0.05$) compared to the 5th day (Table 2).

When the BAL OSI values of the patients were compared, we found that there was a statistically significant decrease on the 3rd day compared to the 1st day ($p<0.05$). BAL OSI values were found to be increased on the 5th day in a statistically insignificant way ($p>0.05$) compared to the 1st day, and statistically significantly ($p<0.01$) compared to the 3rd day. BAL OSI values were found to increase statistically significantly on the 7th day ($p<0.001$) (Table 2).

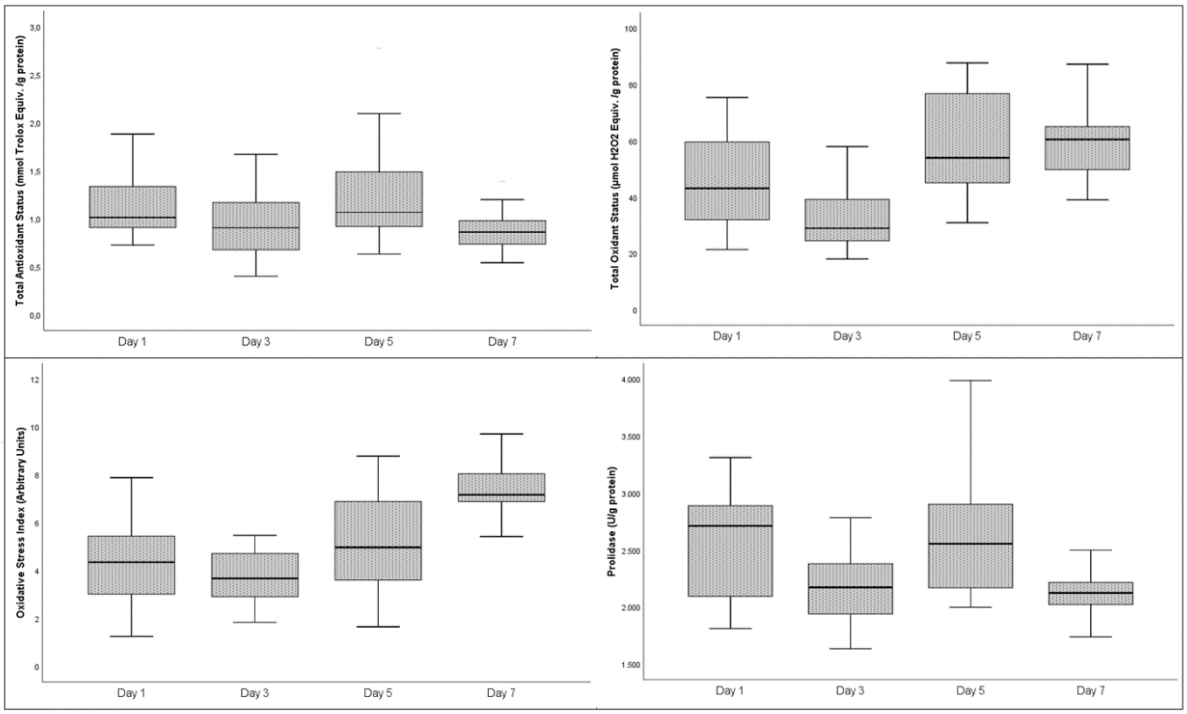
When the BAL prolidase values of the patients were compared, it was found to be statistically significantly lower on the 3rd day compared to the 1st day ($p<0.05$). On the fifth day, a statistically insignificant ($p>0.05$) increase was found in the BAL prolidase level compared to the 1st day, and a statistically significant ($p<0.05$) increase was found compared to the 3rd day. The decrease in BAL prolidase levels on the 7th day was statistically significant compared to the 1st and 7th days ($p<0.001$), but it was not statistically significant compared to the 3rd day ($p>0.05$) (Table 2) (Figure 2).

Table 2. BAL prolidase and BAL Oxidant/Antioxidant parameters of the patients

	BAL Day 1 (n= 26)	**BAL Day 3 (n= 26)**	**BAL Day 5 (n= 26)**	**BAL Day 7 (n= 26)**	***P***
Prolidase (U/L)	2560±434	2203 ± 410^{a*}	2590 ± 481^{d*}	2122±260^{c***,f***}	0.003
Total Oxidant Level (µmol H_2O_2 Eqv./L)	45.84±15.79	32.50 ± 10.99^{a***}	57.36 ± 18.08^{b**,d***}	60.62±14.93^{c***,e***}	<0.001
Total Antioxidant Level (mmol Troloks Eqv./L)	1.14±0.34	0.93 ± 0.30^{a*}	1.22 ± 0.45^{d**}	0.86±0.19^{c**,f***}	0.028
Oxidative Stress Index (AU)	4.25±1.60	3.65 ± 1.04^{a*}	5.17 ± 2.06^{d**}	7.17±1.55^{c***,e***,f***}	<0.001

a: 1 to 3; b: 1 to 5; c: 1 to 7; d: 3 to 5; e: 3 to 7; f: There is a significant difference between the 5th and 7th day. ***: p≤0.001; **: p≤0.01; *: p≤0.05

Figure 2. BAL on the 1st. 3rd. 5th and 7th days of the patients; Difference. distribution and standard deviations between TAS. TOS. OSI. Prolidases



Discussion

Mechanical ventilation therapy can also cause undesirable conditions in the lungs in correlation with the current state of the patient's lungs and the length of stay in the ventilator. Studies show that MV causes oxidative stress in the lung tissue (1,9).

One of the undesirable conditions associated with MV is Ventilator Associated Diaphragm Dysfunction (VIDD). It has been shown that MV-related markers and oxidative stress increase in the diaphragm and there is damage due to oxidation. Mitochondrial abnormalities in the diaphragm during and after MV are seen as evidence of diaphragmatic damage due to ROS (10-18). It is thought that increased lipid peroxidation and protein oxidation cause changes in complexes II, III, IV in the electron transport chain and cause mitochondrial depression and damage (14).

Limited human studies and extensive animal experiments show that MV can cause intradiaphragmatic muscle fiber damage and atrophy, which can cause diaphragmatic failure in many critically ill patients undergoing MV (16,19,20). There are limited studies suggesting that MV-induced oxidative stress in lung tissue may be an indicator of lung injury. In rabbits with iatrogenic hemorrhagic shock; It has been found that in lung damage due to hemorrhagic shock, ischemia during resuscitation, and reperfusion, cytokine levels and ROS levels in BAL increase in a correlated way, and ROS indicate acute lung injury (21,22).

Diaphragm weakness may occur as the duration of mechanical ventilation used in patients with respiratory failure is prolonged. This is thought to cause prolonged weaning (23). It is thought that mechanical ventilation causes diaphragmatic dysfunction by increasing oxidative stress at the diaphragm level, which prolongs the time it takes patients to leave the ventilator. A compilation on these issues was made by Petrof et al. in the compilation; Diaphragm function has been shown to be an important determinant of weaning in mechanically ventilated patients. Although MV is curative in acute lung injury, it can paradoxically increase lung damage (22-26).

In summary, oxidative stress during mechanical ventilation may possibly be a common cause of diaphragmatic dysfunction. Diaphragm dysfunction can also be considered as a cause of prolonged weaning. However, the target in this regard should be to prevent these processes in particular.

In our study; it was investigated how oxidative stress parameters and prolidase (collagen return marker) levels in blood and BAL were affected depending on the duration of MV in intensive care patients without primary lung pathology, and whether this could be used in the early detection of lung damage. Our study; It is important because it is performed in humans and because prolidase is used to show tissue damage due to MV in the lung (3). A fully automatic colorimetric method developed by Erel (many oxidants, total oxidant capacity and many antioxidants were precisely measured) was used (8,9).

The results we found in the serum examinations in our study; shows that patients who started to receive MV therapy were initially affected only locally (lungs) due to intensive care and MV, but were not yet exposed to oxidative damage systemically. Oxidative damage due to MV becomes more evident systemically after the 3rd day. On the 3rd day, we detected that TOS and OSI were highly elevated in the serum, thus oxidative stress was present, and TAS decreased due to excessive consumption in reaction to this and was insufficient against excessive oxidative stress. On the 5th day, the longer the patients stay in the intensive care unit, the significant decrease in TAS, indicating that oxidative stress in the body and thus oxidative damage continues. However, although the increase in OSI on the 5th and 7th days was statistically significant compared to the 1st day, it was not significant compared to the 3rd day. This shows that the consumed antioxidants try to protect the body against oxidative stress, especially on the 3rd day, the oxidative stress, which is very aggravated, is tried to be tolerated by the body and the oxidant / antioxidant balance is maintained. We think that MV therapy also contributed positively to this situation. However, TOS and OSI levels increase very significantly due to the length of stay in the intensive care unit and the prolongation of oxygen exposure at a higher rate than normal (40%) with MV. However, TAS levels decrease significantly due to long-term excessive use. This suggests that the prolonged stay of patients on mechanical ventilators in the intensive care unit leads to an excessive increase in oxidative stress, thus rendering the body's efforts to maintain the oxidant/antioxidant balance insufficient.

The fact that the levels of prolidase, TOS and OSI were statistically significantly increased on the 3rd day compared to the 1st day blood, indicating that an excessive oxidative stress occurred in the patients. This shows that by causing oxidative damage in biomolecules such as lipids and proteins, collagen turnover is accelerated, and as a result, prolidase activity gradually increases. As a matter of fact, on the 5th day, serum prolidase levels were found to be significantly higher, showing that severe oxidative damage continues at the molecular level. However, prolonged stay in intensive care unit causes oxidative stress to increase gradually. A statistically insignificant increase in prolidase level indicated that the acceleration in collagen turnover continued.

In MV therapy, treatment is usually started with 100% oxygen at the beginning, and it is reduced to the lowest oxygen concentration as soon as possible. In the BAL samples of the patients included in the study, TOS and OSI increase on the 1st day due to the excessive oxygen pressure exposed by mechanical ventilation. Accordingly, TAS (due to the sulfhydryl groups it contains) is high, although not excessive. In addition, we can say that prolidase is also high in the 1st day BAL samples, with the destruction of collagen on the bronchoalveolar epithelial surface due to MV. A significant decrease in BAL prolidase level on the 3rd day (compared to the 1st day) indicates that oxidative damage has occurred in the bronchoalveolar surface (lipids and proteins at the molecular level), which is overexposed to oxygen radicals. The increase of prolidase on day 1 in parallel with the accelerated turn-over with collagen damage also confirms our hypothesis. On the 3rd day, the decrease in oxidative stress at the level of bronchoalveolar tissue, decrease in collagen damage (stabilization of collagen turnover) is confirmed by a decrease in prolidase level. The increase in BAL prolidase at day 5 showed collagen degradation on the bronchoalveolar surface (due to mechanical and oxidative damage). On the 7th day, we observed that the BAL prolidase levels were significantly lower than both the 1st day and the 5th day. In this case; We can assume that the prolongation of the patients' dependence on mechanical ventilators in the intensive care unit depletes the total antioxidants of the bronchoalveolar surface (due to both mechanical and severe oxidative damage). However, as the exposure time increases, the protective feature of the mucus

layer covering the surface epithelium may have entered the adaptation process with cellular mechanisms. As a matter of fact, the low prolidase level (compared to the 5th day) indicates that collagen metabolism can be controlled and the turn-over rate can be stabilized.

In the light of these findings, there is a serious oxidative stress that increases with the duration of MV in patients undergoing MV, and this plays a role in the etiopathogenesis and progression of MV-induced lung injury, so a serious oxidative damage occurs and collagen (one of the most affected proteins) is damaged. We can say that in MV-induced lung injury, collagen degradation increases and collagen turnover rate increases, and therefore prolidase enzyme activity in serum increases. Modified Chinard Method, which is a photometric method for demonstrating prolidase enzyme activity, is not yet used as a routine parameter because it is not automated, reliable but not easily applicable. In addition, we think that oxidative stress and oxidative damage occur due to MV in these patients with high oxidative stress markers. The increase in oxidant substances as well as the decrease in antioxidant capacity may contribute to this process by creating collagen tissue damage.

Conclusion

In our study, serum and BAL oxidative stress parameters and prolidase enzyme were found to be associated with MV duration. As the duration of MV treatment increases, prolidase in serum also increases as an indicator of tissue damage in the lungs. We think that serum prolidase enzyme activity can be used to detect and control MV-induced lung damage at an earlier stage. Further studies are needed to support our results at the molecular level in order to reveal the local mechanical effects of MV more comprehensively.

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Ethical Approval: *Ethical approval: Harran University Ethics Committee "DATE: 12 February 2009, SESSION NO: 02.*

Author Contributions*: ED: Study conception and design, interpretation of data, writing manuscript, data acquisition.*

ZB: Study conception and design ŞY: Analysis and interpretation of data, literature review

HA: Interpretation of data. HÇ: Statistical analysis of data and critical revision of manuscript

MK: Prepared figures and writing manuscript. NA: Review and interpretation of data

Conflict of Interest: *The authors have no conflicts of interest to declare.*

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