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ORIGINAL ARTICLE

Evaluation of Trimethylamine N-Oxide (TMAO) Levels in Blunt Thoracic Trauma: An Experimental Study

Künt Toraks Travmasında Trimetilamin N-Oksit (TMAO) Düzeylerinin Değerlendirilmesi: Deneysel Bir Çalışma

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ABSTRACT

Background/Aim: Thoracic traumas cause life-threatening problems ranging from pulmonary contusion to multi-organ injuries. One of the most common complications of these traumas is acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). It is important to establish a biochemical marker to determine the severity of traumatic injuries and to monitor the inflammatory process. In this study, we aimed to measure the serum concentration of trimethylamine N-oxide (TMAO) and to investigate the diagnostic value of this metabolite in a low (3.31 joules), medium (6.62 joules) and high energy (9.93 joules) model of blunt thoracic trauma in rabbits.
Material and methods: In this study, 27 New Zealand rabbits were divided into four groups: Control, low energy trauma group, medium energy trauma group, and high energy trauma group. Blood samples were collected at 1st , 12th and 24th hours after thoracic trauma.
Results: Statistically significant differences in TMAO levels were found both within and between groups ($p<0.0001$).
Conclusion: TMAO levels increased especially in the first hour following trauma and decreased at 12th and 24th hours compared to the first hour (in moderate and high energy trauma groups). These findings suggest that TMAO levels may be related to the severity and timing of trauma. In ALI resulting from blunt thoracic trauma induced at different energy levels, TMAO levels varied between groups and were associated with the timing and severity of trauma. These findings suggest that TMAO levels may be valuable in evaluating the prognosis of trauma and monitoring the inflammatory process.

Keywords: Acute lung injury; Trimethylamine N-oxide; Biomarker; Trauma

ÖZ

Arka plan/Amaç: Toraks travmaları, akciğer kontüzyonundan çoklu organ yaralanmalarına kadar yaşamı tehdit eden problemlere yol açabilir. Bu travmaların yaygın komplikasyonlarından biri de akut akciğer hasarı (ALI) ve akut solunum sıkıntısı sendromu (ARDS) olarak bilinir. Travmatik yaralanmaların şiddetini belirlemek ve inflamatuar süreci izlemek için biyokimyasal bir belirteç oluşturmak önemlidir. Bu çalışmada, tavşanlarda düşük (3,31 joule), orta (6,62 joule) ve yüksek enerjili (9,93 joule) künt toraks travması modelinde serum Trimetilamin N-oksit (TMAO) düzeylerini ölçmek ve bu metabolitin tanısal değerini araştırmak amaçlanmıştır.
Gereç ve Yöntemler: Bu çalışmada, 27 Yeni Zelanda tavşanı dört gruba ayrıldı: kontrol, düşük enerjili travma grubu, orta enerjili travma grubu ve yüksek enerjili travma grubu. Kan örnekleri toraks travmasından sonraki 1., 12. ve 24. saatlerde alındı.
Bulgular: TMAO düzeyleri açısından hem gruplar içinde hem de gruplar arasında istatistiksel olarak anlamlı farklar bulundu ($p<0.0001$).
Sonuç: TMAO seviyeleri, özellikle travmayı takip eden ilk saatte yükselmekte, 12 ve 24. saatlerde ilk saate kıyasla düşmektedir (orta ve yüksek enerjili travma gruplarında). Bu bulgular, TMAO düzeylerinin travmanın şiddeti ve zamanlamasıyla ilişkili olabileceğini göstermektedir. Farklı enerji seviyelerinde oluşturulan künt toraks travması sonucunda ALI'de, TMAO seviyeleri gruplar arasında değişiklik göstermiş ve travmanın zamanlaması ve şiddetiyle ilişkilendirilmiştir. Bu bulgular, TMAO düzeylerinin travmanın prognozunu değerlendirmekte ve inflamatuar süreci izlemekte değerli olabileceğini düşündürmektedir.

Anahtar Kelimeler: Akut akciğer hasarı, Trimetilamin N-oksit, Biyobelirteç, Travma.

Introduction

Thoracic trauma causes considerable health, social and economic problems and is widespread in society. Severe thoracic trauma accounts for about one third of all trauma patients treated in hospital. These injuries affect an area of the body containing vital organs such as heart and lungs and contribute to 25% of all deaths from traumatic injury (1). Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are conditions of sudden respiratory failure associated with lung fluid accumulation in the lungs due to increased permeability of the membrane between the alveoli and the blood vessels (2). Both ALI and ARDS can occur as a complication of physical injury (3). Trimethylamine N-oxide (TMAO) is a metabolite formed in the human body by the oxidation of trimethylamine by hepatic enzymes (4). TMAO is associated with atherosclerosis and has been the subject of toxicological and clinical interest due to its potential health effects (5, 6). TMAO has been reported to cause atherosclerosis via several mechanisms, including decreased bile acid

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biosynthesis, altered reverse cholesterol transport, foam cell formation, and cholesterol accumulation in macrophages (7). TMAO has also been shown to contribute to heart failure and lead to local and systemic inflammatory reactions (8). In addition, TMAO is associated with the impairment of endothelial nitric oxide (NO) synthase activity, which promotes oxidative stress and inflammation (9). TMAO is associated with triggering chronic inflammatory responses that lead to cerebrovascular disease (10).

In summary, TMAO plays an important role in modulating inflammatory responses, particularly in the context of cardiovascular health, atherosclerosis and other health conditions. Considering that bilateral blunt thoracic trauma is also an inflammatory process, it is likely to be associated with TMAO levels. Nevertheless, there is a lack of research investigating this relationship. It is important to investigate whether TMAO can serve as a biomarker to predict which ALI patients might progress to severe ARDS requiring prolonged ventilator support, and which might develop pulmonary fibrosis and ultimately face death. However, current studies are insufficient for the routine use of biomarkers that indicate the severity of injury thoracic trauma.

The aim of our study was to measure serum TMAO levels and investigate the diagnostic value of TMAO in an experimental model of low, medium, and high energy blunt thoracic trauma in rabbits.

Material And Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of ***, Experimental Medicine Research and Application Center (protocol code 2022-39 and date of approval 30/09/2022) for studies.

Experimental Model

The study was conducted on 27 New Zealand rabbits aged 1 to 2 years, bred at the Experimental Animal Research Center of *** University.

Control group (n=6): No trauma was administered to this group during the experiment. Blood samples were taken after 1st, 12th and 24th hours. As the venous vascular system was not anatomically suitable for blood sampling due to the administration of fluid and medication via the venous route, both the arterial and venous routes were used. After 24 hours, the subjects were sacrificed.

Low energy trauma group (n=7): Low energy trauma (3.31 joules) was inflicted on the rabbits in this group without blood sampling. After the trauma, blood samples were taken after 3 hours as in the control group and the subjects were then sacrificed.

Medium energy trauma group (n=7): Medium energy trauma (6.62 joules) was administered prior to blood collection, and samples were collected at three different time points post-trauma during the experiment. After 24 hours, the subjects were sacrificed.

High energy trauma group (n=7): Blood samples were collected after the application of trauma (9.93 joules). After 24 hours, the subjects were sacrificed.

Anesthesia and Trauma Model

All traumatized subjects were anesthetized with 40 mg/kg ketamine HCl (Ketalar) and 10 mg/kg xylazine HCl (Rompun) administered intramuscularly into the hind leg of the rabbit. The bilateral blunt thoracic trauma model previously used in other studies was modified. The low, medium and high energy trauma groups were subjected to the same model with the addition of a variable weight. Using the bilateral blunt thoracic trauma model, weights of 250 g, 500 g and 750 g were dropped from a height of 0.62 meters onto the lateral wall of the thorax for the low, medium and high energy trauma groups. The resulting energy was calculated using the formula E= mgh (where E is energy, g is gravity at 10 m/s², h is height at 62 cm, and m is the weight dropped at 0.25 kg, 0.50 kg, and 0.75 kg). Accordingly, the energy transferred to the chest wall was determined as 3.31 joules (low energy), 6.62 joules (medium energy), and 9.93 joules (high energy), assuming a frictionless scenario. After 24 hours, the surviving subjects were sacrificed with a sedation dose that was twice that of the original anesthesia (11).

Biochemical analysis

Blood samples taken after at 1st, 12th and 24th hours were stored at -80°C until analysis. To determine the TMAO content, the previously prepared serum samples were thawed at room temperature and analyzed using an LC-MS/MS instrument (ABSciex API 3200 tandem mass spectrometer) in the biochemistry laboratory of the *** University Faculty of Medicine. In brief, each tube received the TMAO isotope, followed by the addition of 100% methylene hydroxide as a precipitant. After all tubes were gently shaken for 30 seconds, they were centrifuged at 14,000 rpm for 10 minutes. The supernatant of the samples was then transferred to clean tubes. The tubes were evaporated under nitrogen gas at 28°C. The samples were reconstituted with 100% water. After centrifugation at 4,500 rpm for 10 minutes, the supernatant was collected and transferred to vials compatible with the LC-MS/MS instrument. The TMAO concentrations in the samples were determined against the TMAO standard in the LC-MS/MS instrument using two mobile phases. The results obtained were statistically analyzed.

Statistical analysis

All statistical analyses were performed with the software R version 3.6.0. The normality of the data was checked using the Shapiro-Wilk normality test. The variables were expressed as mean ± standard deviation. One-way analysis of variance or the Kruskal-Wallis test was used to compare the measured parameters between the groups. The Tukey HSD test or the DSCF post hoc test with Bonferroni correction was used for the pairwise comparison of the parameters. In addition, repeated measures analysis of variance or Friedman test was used for comparison of parameters

between measurement time points in each group and pairwise comparisons were performed using Bonferroni confidence intervals or Durbin-Conover post hoc tests. The statistical significance level was set at p<0.05.

Results

The study included 27 rabbits divided into four groups: a control group (n=6), a low energy trauma group (n=7), a medium energy trauma group (n=7), and a high energy trauma group (n=7).

In terms of temporal comparison within groups, serum TMAO levels decreased significantly at 12th hour (107.47 ± 14.00) and at 24th hour (89.97 ± 2.34) in the medium energy trauma group compared to levels at 1st hour (176.29 ± 7.13) (p<0.0001). Similarly, TMAO levels decreased significantly in the high energy trauma group at 24th hour (172.86 ± 20.09) compared to 1st hour (310.29 ± 50.23) and 12th hour (241.00 ± 26.64) after trauma (p=0.028) (Table 1).

Comparison between the groups: At the 1st hour, TMAO levels were lower in the low energy trauma group than in the control group, the medium energy trauma group, and the high energy trauma group (p<0.0001). At 12th and 24th hours, TMAO levels were significantly lower in the low and medium energy trauma groups than in the control group (p<0.0001). In addition, TMAO levels were higher in the high energy trauma group than in the medium energy trauma group (p<0.0001) (Table 1). Figure 1 also summarizes the change in TMAO levels within the groups as a function of time.

Table 1. Laboratory values of the experimental groups depending on time and trauma level.

Biomarker	Groups	Time			p-value (Within groups)
		1st hour	12th hour	24th hour	
TMAO (ng/ml)	Control group	201.33 ± 10.99^{a}	162.00 ± 5.81^{a}	211.67 ± 19.28^{a}	0.070
	Low energy trauma group	126.11 ± 10.63^{b}	105.34 ± 9.13^{b}	115.66 ± 13.63^{b}	0.410
	Medium energy trauma group	176.29 ± 7.13^{a}	107.47 ± 14.00^{Ab}	89.97 ± 2.34^{Bb}	<0.001
	High energy trauma group	310.29 ± 50.23^{a}	241.00 ± 26.64^{a}	172.86 ± 20.09^{BCa}	0.028
	p-value (Between groups)	<0.001	<0.001	<0.001	

Data were expressed as mean ± standard error of the mean.

A shows comparison 1st vs. 12th hour.; B shows comparison 1st vs. 24th hour.; C shows comparison 12th vs. 24th hour.

Different small superscripts letters in each column indicate that statistically significant difference between two groups after pairwise comparisons.

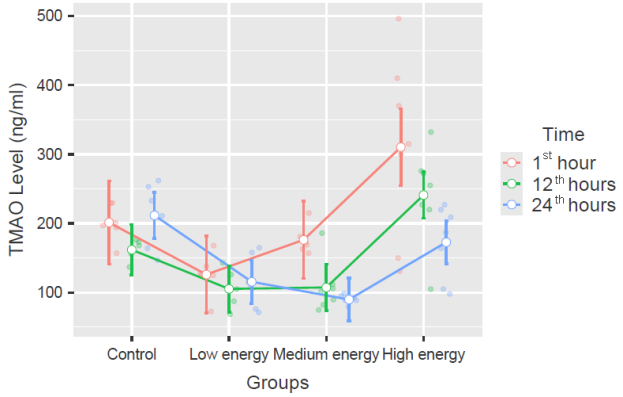

Figure 1. The graph shows the changes in the TMAO (ng/ml) for four different groups (control, low energy trauma group, medium energy trauma group, and high energy trauma group) according to three different times (1st , 12th , and 24th hours)

Discussion

This study is the first to evaluate the relationship between trauma severity, timing of trauma, and TMAO. The results support the hypothesis that TMAO levels may be useful in assessing the prognosis of trauma and monitoring the inflammatory process.

Thoracic trauma causes life-threatening problems ranging from pulmonary contusion to multi-organ injury, while ALI and ARDS are common complications of traumatic injury (12). The aim of our study was to measure TMAO levels to determine the lung damage caused by low, medium, and high blunt thoracic trauma in rabbits and to investigate the diagnostic and prognostic value of TMAO. A recently developed trauma model was used to investigate the degree of lung injury and the natural pulmonary inflammatory response in rabbits (11). This model is clinically similar to lung injury after blunt thoracic trauma.

Pulmonary contusion, a common consequence of blunt trauma to the chest, is characterized by localized pulmonary injury and inflammation. The mechanical forces applied during trauma trigger oxidative stress, the formation of reactive oxygen species, leukocyte infiltration and the release of inflammatory cytokines. This cascade of events can induce a systemic inflammatory response and impair endothelial function while contributing to the initiation and potentiation of the inflammatory process (13, 14). Tissue damage and inflammation caused by trauma can affect the gut microbiota and increase TMAO production (15). TMAO is a biologically active molecule that plays a role in the development of diseases such as thrombosis, stroke, heart disease, type 2 diabetes, obesity, especially atherosclerosis (5, 16). In the context of vascular health, TMAO has been associated with the exacerbation of vascular inflammation and oxidative stress, leading to endothelial dysfunction and atherosclerosis (4). In the study by Brunt et al., TMAO was shown to cause age-related endothelial dysfunction through oxidative stress (17). Sun et al. have shown that TMAO significantly triggers oxidative stress, dose- and time-dependent release of inflammatory cytokines, and inhibition of endothelial NO synthase and NO production. Therefore, increased TMAO levels are associated with decreased NO bioavailability as an inhibitor of NO

synthase and thus disease severity (18). According to the data, TMAO levels were elevated in the first hour after trauma and in the medium and high energy trauma groups. No significant difference was found in the low energy trauma group. The lack of a significant difference can be attributed to the low intensity of the trauma applied, which resulted in the absence of the expected effect in the animals. Considering the studies, since endothelial dysfunction caused by NO depletion and the concomitant inflammatory process may lead to the release of inflammatory markers in the body, an increase in TMAO was observed in relation to the severity and timing of the trauma. Administration of low dose NO attenuated oxidative stress and inflammatory lung injury in the ALI rabbit model (19).

In trauma research, there is a growing interest in understanding the molecular and metabolic changes that occur after traumatic events. Metabolic profiling has revealed changes in urinary TMAO concentrations following surgical trauma, indicating its potential as a biomarker of surgical stress and trauma (20). Ina addition, the inflammatory response following severe traumatic brain injury has been shown to modulate the kinetic profile of inflammatory markers, highlighting the complex interplay between trauma and the immune system (21). TMAO, a microbial metabolite from the gut, is known to play an important role in immune responses, inflammatory processes and various diseases (22, 23).

TMAO has been associated with the promotion of vascular inflammation and endothelial dysfunction, which are critical components of the pathophysiologic response to ALI (24). In addition, TMAO has been associated with cardiovascular risk and unfavorable outcomes in patients with acute myocardial infarction and stroke, indicating its potential influence on systemic inflammatory responses and tissue damage (25).

The effects of TMAO on inflammation extend to various aspects of vascular health, gut microbiota, and regulation of inflammatory mediators, highlighting its potential as a key factor in the pathogenesis of inflammation-related diseases (26). Although there are no studies investigating this relationship, it is hypothesized that there is an association between TMAO levels and bilateral blunt chest trauma, as it is an inflammatory process. And the results showed significant differences in TMAO levels between different energy levels and time points, suggesting a possible relationship with the severity and progression of the trauma. In particular, in the medium and high energy level groups, the elevated TMAO levels in the first hour after trauma possibly indicate an acute phase response and the severity of the injury. The subsequent decrease after 12th and 24th hours can indicate the subsiding of the acute response. TMAO has the potential to serve as a biomarker for predicting various outcomes in patients with ALI, such as the development of severe ARDS, the need for prolonged ventilatory support, the occurrence of pulmonary fibrosis and ultimately mortality. However, the study evidence is not yet sufficient for the routine use of the TMAO biomarker to indicate the severity of injury following chest trauma. Future studies focusing on the interaction between TMAO and blunt chest trauma are needed to improve our understanding of potential biomarkers associated with this type of trauma.

Limitations of the study: TMAO may be associated with the severity of trauma and the patient's prognosis in the early phase of trauma. While it is hypothesized that it can be used as a biomarker, the fact that we do not have enough information on the prognosis of the animals included in the study, as the study was terminated early (24 hours after trauma), is considered an important limitation. Further studies on this topic are needed.

Conclusion

TMAO has been associated with numerous pathophysiologic conditions, most notably atherosclerosis, and it is likely that it is also associated with trauma. Blunt thoracic trauma is an important cause of injury and death, often leading to various thoracic complications and concomitant injuries. Therefore, biomarkers such as TMAO are valuable tools for understanding the physiological and psychological effects of trauma. We believe that the determination of TMAO levels in our study can help to evaluate the prognosis of trauma and monitor the inflammatory process. We believe that further studies should be conducted to substantiate these ideas. To our current knowledge, this is the first study in this field.

Declarations

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Declaration of Competing Interest

The authors declare that they have no conflict of interest..

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None.

Data Availability

The data sets generated during or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Selçuk, Experimental Medicine Applications and Research Centre (protocol code 2022-39 and date of approval 30/09/2022) for studies.

Author Contributions

Conception: B.O., A.B., H.K., F.A., H.V., Data Collection and Processing: B.O., A.B., H.K., Design: : F.S., F.A., B.O., H.V., Supervision: B.O., Analysis and Interpretation: F.S., F.A., M.K.K., Literature Review: F.S., F.A., H.V., Writer:

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