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ORIGINAL ARTICLE

Diagnostic Value of Serum Clara Cell Secretory Protein Level in an Experimental Blunt Chest Trauma Model

Deneysel Künt Toraks Travması Modelinde Serum Clara Hücresi Salgı Proteini Düzeyinin Tanısal Değeri

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ABSTRACT

Objective: Blunt chest trauma is a significant clinical problem leading to injury of the lungs that may be fatal. Experimental blunt chest trauma is well established in animal models. This study aimed to investigate whether Clara cell secretory protein (CC16) can be a biomarker in an experimentally created blunt chest trauma model.
Material and Methods: A total of 30 rabbits were used in our study. A modified bilateral blunt thoracic trauma model was used to produce different levels of lung contusion. We divided the rabbits into four groups according to the energy level at which blunt thoracic trauma was applied. Blood samples were taken from the control and trauma groups to evaluate CC16 levels at 0, 12, and 24 hours.
Results: The CC16 levels measured at the start of the experiment were significantly lower in the control and low-energy groups compared to the medium- and high-energy groups (p =.002). While there was a significant difference in CC16 levels measured at the 12th hour (p =.004), no significant difference was found among the groups at the 24th hour. Upon analyzing the change in CC16 levels over time within the groups, we observed that CC16 levels decreased from 0–12 hours and subsequently increased after the 12th hour. Histopathologically, we observed that the level of contusion increased in proportion to the severity of trauma across the different groups.
Conclusion: With the designed platform, we created a reproducible experimental model of pulmonary contusion from blunt thoracic trauma in rabbits. Increased levels of CC16 following a lung contusion could serve as a foundation for clinical decision-making. Thus, CC16 has the potential to serve as a rapid and simple biochemical indicator for acute traumatic lung injury.

Keywords: Experimental, Blunt chest trauma, Biomarker, CC16

ÖZ

Amaç: Künt toraks travması akciğerlerde ölümcül olabilen hasara yol açan önemli bir klinik problemdir. Deneysel künt toraks travması hayvan modelleriyle iyi bir şekilde ortaya konmuştur. Bu çalışmada deneysel olarak oluşturulan künt toraks travması modelinde Clara hücresi salgı proteininin (CC16) biyobelirteç olarak kullanımının araştırılması amaçlandı.
Gereç ve Yöntemler: Çalışmamızda toplam 30 adet tavşan kullanıldı. Farklı düzeylerde pulmoner kontüzyon oluşturmak için modifiye edilmiş iki taraflı künt toraks travma modeli kullanıldı. Künt toraks travmasının uygulandığı enerji düzeyine göre tavşanları dört gruba ayırdık. Kontrol ve travma gruplarından 0, 12 ve 24. saatlerde CC16 düzeylerini değerlendirmek için kan örnekleri alındı.
Bulgular: Deneyin başlangıcında ölçülen CC16 seviyeleri kontrol ve düşük enerji gruplarında orta ve yüksek enerji gruplarına göre anlamlı derecede düşüktü (p =.002). 12. saatte ölçülen CC16 düzeyleri arasında anlamlı fark bulunurken (p=0,004), 24. saatte gruplar arasında anlamlı fark bulunamadı. Grupların CC16 düzeylerinin zaman içindeki değişimini incelediğimizde CC16 düzeylerinin 0-12. saatlerde azaldığını, 12. saatten sonra ise arttığını gözlemledik. Histopatolojik olarak farklı gruplarda travmanın ciddiyeti ile orantılı olarak kontüzyon seviyesinin arttığını gözlemledik.
Sonuç: Tasarlanan platformla tavşanlarda künt toraks travmasından kaynaklanan pulmoner kontüzyonun tekrarlanabilir deneysel bir modelini oluşturduk. Pulmoner kontüzyonu takiben artan CC16 seviyeleri, klinik karar vermenin temelini oluşturabilir. Bu nedenle CC16, akut travmatik akciğer hasarı için hızlı ve basit bir biyokimyasal gösterge olarak kullanılma potansiyeline sahiptir.

Anahtar Kelimeler: Deneysel, Künt toraks travması, Biyobelirteç, CC16

Introduction

Blunt injury to the chest may effect any or all components of the chest wall and chest cavity. These components include the bony skeleton, lungs and pleura, tracheobronchial tree, esophagus, heart, great vessels of the chest, and diaphragm. (1) Blunt chest trauma is directly responsible for 25% of all trauma deaths. (2) After blunt chest trauma, life-threatening complications, such as airway obstruction, severe pulmonary contusion, tension pneumothorax, and massive hemothorax, may be observed during primary examination and require evaluation and treatment. A pulmonary contusion is bruising of the lungs usually caused by blunt chest trauma. As a result of damage to the capillaries, blood and other fluids accumulate in the lung tissue. The excess fluid interferes with gas exchange, potentially leading to hypoxia. (3) Pulmonary contusion has been identified as a risk factor for acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). 20% of these patients may develop ALI or ARDS. (4) Trauma-associated ALI/ARDS has a lower mortality than ALI/ARDS associated with sepsis or other clinical risk factors, such as pneumonia, pancreatitis, or aspiration, possibly implying a different mechanism or pathological basis for the disease. (5) The pathophysiology of pulmonary contusion

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includes loss of alveolar–capillary membrane integrity and pulmonary edema, excessive transepithelial neutrophil migration, and release of proinflammatory and cytotoxic mediators, ventilation/perfusion mismatching, increased intrapulmonary shunting, and a loss of compliance. (6-9) Some biomarkers in the epithelium and endothelium involved in the inflammatory process may play a role in predicting the morbidity and mortality of traumatic lung injury. Clara cell secretory protein (CC16) is a protein secreted throughout the tracheobronchial tree and particularly in terminal bronchioles where Clara cells are localized. The functions of Clara cells mainly include protection of the respiratory tract. Clara cells act as stem cells in the repair of bronchial epithelium, have high xenobiotic biotransformation capacity, and secrete various substances with important biological activities. CC16 has anti-inflammatory and immunomodulatory functions in the lung, and the serum CC16 level reflects changes in inflammation, changes in epithelial integrity, and airway inflammation. (10, 11) CC16 has been investigated as a biomarker of lung epithelial damage in numerous disease states, including ALI/ARDS, chronic obstructive pulmonary disease, asthma, occupational or environmental lung injury, tobacco use, pulmonary fibrosis, and sarcoidosis. (12, 13) We hypothesized that serum CC16 levels reflect the severity of trauma-associated traumatic pulmonary contusion and may be useful in diagnostic assessment.

Material and Methods

Study Design

The study protocol was approved by the Ethics Committee of the Selçuk University Experimental Medicine Research and Application Center. The National Institutes of Health Guide for Care and Use of Laboratory Animals was followed for all experiments. New Zealand rabbits (age 1–2 years) were randomized into four groups: a control group (n = 6), a low-energy group (Group A) (n = 8), a medium-energy group (Group B) (n = 8), and a high-energy group (Group C) (n = 8). Anesthesia was induced with xylazine hydrochloride (10 mg/kg intramuscular) and ketamine (40 mg/kg intramuscular). By adding a changeable weight feature to the modified bilateral blunt thoracic trauma model, low-, medium-, and high-energy trauma was achieved with the same model. The bilateral blunt thorax trauma model involved dropping 250 g, 500 g, and 750 g weights from a height of 0.62 meters onto the bilateral thorax side walls for low-, medium-, and high-energy trauma, respectively, on subjects who were sedated and supine on a flat platform. The resulting energy was determined using the formula E = mgh, where E = energy, g = gravity (10 m/s2), h = height (0.62 m), and m = dropped weight (0.25 kg, 0.50 kg, or 0.75 kg). The energy transferred to the chest wall was determined to be 3.31 J (low energy), 6.62 J (medium energy), and 9.93 J (high energy), respectively, given the 0.62-m height and weights of 250 g, 500 g, and 750 g utilized. The frictional force was disregarded. Following the application of trauma, blood samples were collected from the subjects 0, 12, and 24 hours after the experiment to measure their CC16 levels. Subjects with apnea detected during the 24-hour observation period were sacrificed after asystole was detected by electrocardiography and peripheral oxygen saturation monitoring.

Biochemistry

Serum levels of CC16 (MyBioSource lot no. MBS036950) were determined with the enzyme-linked immunosorbent assay (ELISA) technique. Both the intra-assay and inter-assay coefficients of variation were less than 15%. The minimum detectable concentration was 0.625 ng/ml.

Histology

Twenty-four hours after blunt lung trauma was induced, the same anesthetic agents were administered, and the animals were decapitated. Following macroscopic evaluation of the lung tissue samples, part of the lung tissue was dissected for immunohistopathological testing. The dissected tissues were preserved by immersing them in a freshly prepared solution of 4% paraformaldehyde in PBS for 24 hours at a temperature of +4 °C. Prior to being implanted in the cryomatrix, they were immersed in a 30% sucrose solution at a temperature of +4 °C for one night. Sections were cut from the trauma area using a cryostat device (Leica RM2125 RT) with a thickness of 4 µm at a temperature of −25 °C and then placed onto poly-L-lysine slides. They were marked with a terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) Andy FluorTM 488 Apoptosis Detection Kit (ABP Biosciences, catalog no. a050, lot no. AB2150A2) in accordance with the protocol. As directed by the protocol, both positive and negative controls were also included. An Olympus BX51 trinocular fluorescence microscope equipped with the proper filters (for DAPI staining and the TUNEL kit) was used to evaluate the images. A DP72 camera with a 40× objective magnification was used to capture digital images of six random regions. TUNEL-labeled cells and DAPI-labeled nuclei were counted with the ImageJ program (National Institutes of Health, Bethesda, Maryland, USA), and the apoptotic index ([TUNEL positive cell number/DAPI positive nuclei] × 100) was utilized to evaluate the TUNEL labeling.

Statistical Analysis

Descriptive statistics of continuous variables were obtained for the study and control groups. The Shapiro–Wilk normality test was applied to assess the association between trauma group and degree of contusion. Due to the absence of a normal distribution, the degree of contusion was assessed among trauma groups using the Kruskal–Wallis test. In addition, to account for variation between and within the various trauma groups (repeated plasma samples at 0, 12, and 24 hours), model-fitting procedures were implemented. Repeated measures analysis was conducted using statistical software (R, version 3.0.2, R Foundation for Statistical Computing, Vienna, Austria). (14) For comparisons among multiple groups, the Kruskal–Wallis test was utilized, while the Mann–Whitney U test was applied for post hoc analyses. The effects of trauma severity on CC16 levels were assessed using logistic regression. The criterion for statistical significance was $p \leq .05$.

Results

Of the 30 New Zealand rabbits included in the study, three in Group C died at 14, 20, and 21 hours after trauma, respectively. Blood samples were obtained and analyzed at 0 and 12th hours from 3 died subjects in Group C. However, due to the unavailability of blood samples at the 24th hour, these subjects were excluded at that time point, and the analysis was subsequently conducted on the remaining 5 subjects.

CC16 Levels in Plasma

Both the control group and Group A exhibited significantly lower CC16 levels at 0 hours than Group B and Group C (p =.002). Furthermore, a significant difference was noted between Group B and both the control group and Group A with regard to the CC16 levels measured at the 12-hour mark (p =.004). However, CC16 measurements at the 24-hour mark did not significantly differ across the groups (p =.1) (Table 1).

Table 1. Comparison of CC16 levels and degrees of contusion of the trauma and control groups measured at the same time points (mean ± SD)

Subject	CC16 (0 h)	CC16 (12 h)	CC16 (24 h)	Contusion
Control group	1.9 ± 1.3^{ab}	1 ± 0.3^{a}	1.7 ± 1.5^{a}	1.8 ± 0.7^{a}
Group A	1.8 ± 1.4^{a}	0.8 ± 0.4^{a}	2.3 ± 1.2^{a}	2.7 ± 0.6^{ab}
Group B	6.7 ± 4.6^{c}	2.2 ± 1.2^{b}	2.3 ± 1.2^{a}	4.4 ± 2.7^{ab}
Group C	5.2 ± 2.1^{bc}	4.5 ± 3.6^{ab}	4.8 ± 1.8^{a}	8.5 ± 6.5^{b}
P-value	.002	.004	.1	.008

*Data reported as mean ± SD. Any groups with different superscripts indicate statistically significant differences ($P < .05$).

CC16: Clara cell secretory protein

Upon analyzing the change in CC16 levels among the groups over time, we observed that there was a decrease in CC16 levels between 0 and 12 hours, followed by an increase after 12 hours (Figure 1). The control group, which did not receive any intervention, did not show any substantial variation in terms of CC16 levels at 0, 12, or 24 hours. In Group A, the decrease in CC16 level between 0 and 12 hours was not statistically significant, but there was a significant increase after 12 hours. In Group B, the CC16 level decreased significantly between 0 and 12 hours, and the increase after 12 hours was not significant. In Group C, the CC16 level decreased between 0 and 12 hours and increased after 12 hours, but this was not statistically significant (Table 2).

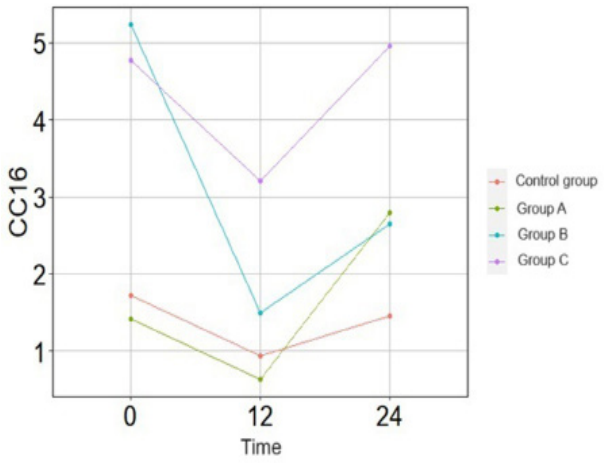

Figure 1. Change over time of the CC16 levels of the control and trauma groups

Table 2. Comparison of the change over time in CC16 levels of the control and trauma groups measured at 0, 12, and 24 hours (mean ± SD)

Subject	CC16 (0 h)	CC16 (12 h)	**CC16 (24 h)**	P-value
Control group	1.9 ± 1.3^{a}	1 ± 0.3^{a}	1.7 ± 1.5^{a}	.8
Group A	1.8 ± 1.4^{ab}	0.8 ± 0.4^{a}	2.3 ± 1.2^{b}	.01
Group B	6.7 ± 4.6^{a}	2.2 ± 1.2^{b}	2.3 ± 1.2^{b}	.005
Group C	5.2 ± 2.1^{a}	4.5 ± 3.6^{a}	4.8 ± 1.8^{a}	.2

*Data reported as mean ± SD. Any groups with different superscripts indicate statistically significant differences ($P < .05$).

CC16: Clara cell secretory protein

Histopathology

Histopathological analysis revealed substantial variation in contusions across the experimental groups, with the degree of contusion rising with increasing trauma severity (Figure 2).

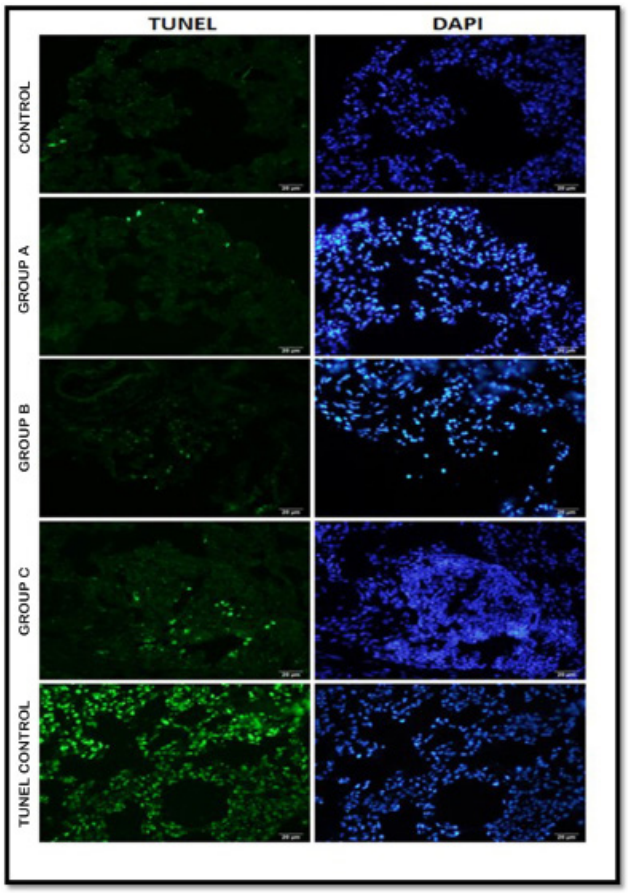

Figure 2. TUNEL-labeled cells and DAPI-labeled nuclei for evaluation of TUNEL labeling of rabbit subjects. TUNEL Positive Cells (Green) in lung sections. Cell nuclei are labeled with DAPI (Blue). (40X magnification, scale bar:20 µm). The lowest TUNEL positive cells were in Control group. TUNEL positive cells of high energy group were higher than low and medium energy groups.

Discussion

Blunt chest trauma represents one of the most critical injuries in patients with multiple injuries. (15) Blunt chest injuries result in significant admission rates to emergency departments, disability, and mortality throughout the world. Severe blunt chest trauma is considered an important trigger of the systemic inflammatory response that occurs after injury. Reported mortality rates of blunt chest injuries vary between 4 and 60%. (16) Currently, none of the available markers, alone or in combination, appear specific and sensitive enough to be considered primary diagnostic tools for the development of respiratory complications. The exact mechanism of most biomarkers and their role in the development of acute traumatic lung injury in trauma patients remain to be elucidated. Prospective clinical studies or advanced statistical approaches may be needed to understand the role of biomarkers in acute traumatic lung injury. In addition, further studies with larger case numbers, preferably with multicenter approaches, are needed. (17)

The purpose of our study was to track changes in serum CC16 concentration following a traumatic lung contusion and to examine the connection between the severity of damage and this condition. CC16 is a specific type of protein that is mostly released by Clara cells located in the distal region of the epithelium of the lung's airway. (18) Damage to Clara cells or alterations in the permeability of the alveolar epithelium affects the synthesis and secretion function of CC16, which is essential for diagnosis of the disease process. (19) During traumatic events, changes such as damage to lung epithelial cells and barrier permeability occur immediately. These changes allow small molecules on the surface of the alveolar epithelium to enter the bloodstream more quickly, depending on the concentration gradient. Therefore, blood concentrations peak rapidly, and CC16 concentrations may be maintained at a high level due to changes in the synthesis and secretory functions of damaged cells. These physical characteristics give the measurement of CC16 levels a clear advantage over imaging in the clinical diagnosis of traumatic pulmonary contusion as a quick and accurate biomarker of lung damage. CC16 is regarded as a sensitive biomarker of acute traumatic lung injury, and changes in CC16 concentration in serum provide a useful reference value for the diagnosis of post-traumatic lung disease. (20, 21) CC16 is efficiently eliminated from the bloodstream through glomerular filtration, resulting in a relatively short half-life of around 2–3 hours (13, 22). Our study found a consistent decrease in CC16 levels within all groups with lung contusion during the first 12 hours, followed by an increase after the 12-hour mark. Furthermore, CC16 serum concentrations in patients with lung contusion were significantly higher than those of the healthy control group, indicating a specific reference value for the diagnosis of lung contusion (Table 1).

The severity of the injury is determined by the volume of the lung contusion. The CC16 level rises very quickly in the blood after contusion, as evidenced by the fact that it was significantly higher in the medium-energy and high-energy trauma groups at the onset of trauma. Furthermore, the decrease in the CC16 level until 12 hours and the subsequent increase after 12 hours may reflect the diurnal change in serum level caused by changes in lung permeability as a result of the accumulation of massive proteins, red blood cells, inflammatory cells, fibrin, and cellular debris after blunt chest injuries. (23, 24) In our study, the severity

of pulmonary traumatic contusion was positively correlated with the serum concentration of CC16; consequently, the higher the concentration of CC16 at the beginning of the experiment, the higher the lung contusion volume. Histopathological analysis of the contusions developed revealed significant differences among the experimental groups in this study; furthermore, as predicted, the degree of contusion increased with the severity of the trauma (Figure 1, Table 1). CC16 may have a reference value for diagnosing and monitoring the severity of a traumatic pulmonary contusion. Furthermore, the rapidity and simplicity of CC16 detection can reduce the need for repeated computed tomography scans on patients, thus indirectly reducing the patient's exposure to radiation and undesirable harm. As a result, CC16 may serve as a biomarker to aid clinical diagnosis and assessment of the severity of traumatic pulmonary contusion, thereby offering a straightforward and effective reference basis for clinical treatment decisions.

Limitations of the present study include the short period of time (24 hours) studied after blunt chest trauma. Therefore, long-term plasma CC16 levels and histopathologic findings were not evaluated. In future studies, it would be useful to evaluate these parameters over longer periods of time after trauma. In addition, the number of rabbits studied was small, especially because three rabbits died, so further study with a larger sample is justified.

In conclusion, traumatic pulmonary contusion can cause severe hypoxic respiratory failure, leading to significant morbidity and mortality. This study has established a useful model for examining CC16 levels in New Zealand rabbits with blunt chest trauma. Our hypothesis was that CC16 may be a new important biomarker of traumatic pulmonary contusion. The present study showed that CC16 may indeed be useful in the diagnosis of traumatic pulmonary contusion. Increased CC16 concentration in patients admitted due to thoracic trauma is an important reference for the presence of pulmonary contusion and the possibility of worsening.

Authors' contributions

Kara H, Bayır A, Bayraktar YŞ and Can A participated in the conception and design of the study; Ak A, Öztürk B, Ünlükal N and Eseroğlu M accessed the data and established the data file; Öztürk B, Kara H, Bayraktar YŞ, Akbuğa F analyzed and interpreted the data; Akkuş A, Bayır A and Eseroğlu M conducted literature searches; Kara H, Ünlükal N, Ak A and Can A drafted the manuscript; Akbuğa F, Bayraktar YŞ and Akkuş A revised the manuscript. All the authors read and approved the final manuscript.

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