RESEARCH ARTICLE

F.U. Med.J.Health.Sci.
2024; 38 (3): 213 - 220
http://www.fusabil.org

# Can CC16 Levels in Serum and TGF-$\beta_1$, Caspase-3, Caspase-8, and Caspase-9 Levels in Bronchoalveolar Lavage Fluid Be Early Markers in Premature Patients with Bronchopulmonary Dysplasia?*

**Mehtap DURUKAN TOSUN** [1, a]
**Sefika ALDAŞ** [1, b]
**Necmiye CANACANKATAN** [2, c]
**Erdal TAŞKIN** [3, d]
**Denizmen AYGÜN** [3, e]

[1] Mersin City Education and Research Hospital, Department of Pediatrics, Mersin, TÜRKIYE

[2] Mersin University, Faculty of Pharmacy, Department of Biochemistry, Mersin, TÜRKIYE

[3] Fırat University, Faculty of School of Medicine, Neonatology Department of Pediatrics, Elazig, TÜRKIYE

[a] ORCİD: 0000-0002-4041-2777
[b] ORCİD: 0009-0006-9222-6163
[c] ORCİD: 0000-0003-3863-962X
[d] ORCİD: 0000-0001-6387-1741
[e] ORCİD: 0000-0002-6450-9282

**Received** : 27.05.2024
**Accepted** : 16.07.2024

**Correspondence Address**

**Mehtap DURUKAN TOSUN**
Mersin City Education and Research Hospital, Department of Pediatrics, Mersin – TURKIYE

**mehtap.durukan@gmail.com**

**Objective:** Advances in neonatal intensive care have increased the survival rate of premature infants, but the incidence of bronchopulmonary dysplasia (BPD) has also risen. Early markers for BPD are still unclear. This study investigates the relationship between BPD development and levels of clara cell secretory protein 16 (CC16), Transforming growth factor-$\beta_1$ (TGF-$\beta_1$), and caspase-3, caspase-8 and caspase-9.

**Materials and Methods:** On days 1, 3, 14, and 28 of life, bronchoalveolar lavage fluid and serum samples were obtained from infants born before 32 weeks gestation and intubated for respiratory distress. 41 cases, including 21 neonates with BPD and 20 without, were included.

**Results:** CC16 levels were consistently higher in the BPD group, peaking on day 3, with significant differences on days 14 and 28 ($p<0.05$). TGF-$\beta_1$ was highest on day 1 in the BPD group, decreasing by day 3, but remained higher than in controls ($p<0.05$). Caspase levels were similar on day 1 but significantly higher in the BPD group on day 3. On day 14, all caspase levels decreased, with caspase-3 continuing to decrease on day 28 while caspase-8 and caspase-9 rose again. Increased uncontrolled apoptosis, particularly through the extrinsic pathway, was linked to BPD development.

**Conclusion:** CC16, TGF-$\beta_1$, and caspases are potential early markers for BPD and could guide new treatment modalities.

***Key Words:*** *Bronchopulmonary dysplasia, CC16, TGF-$\beta_1$, caspases*

## Bronkopulmoner Displazi Gelişen Prematüre Bebeklerde Serum CC16 ve Bronkoalveolar Lavaj Sıvısında TGF-$\beta_1$, Kaspaz-3, Kaspaz-8, Kaspaz-9 Düzeyleri Erken Belirteç Olabilir mi?

**Amaç:** Yenidoğan yoğun bakımındaki gelişmeler prematüre bebeklerin hayatta kalma oranını artırmıştır, ancak bronkopulmoner displazi (BPD) insidansı da yükselmiştir. BPD için erken belirteçler hala belirsizdir. Bu çalışmada BPD gelişimi ile clara hücre salgı proteini 16 (CC16), transforming growth factor-$\beta_1$ (TGF-$\beta_1$) ve kaspaz-3, kaspaz-8, kaspaz-9 düzeyleri arasındaki ilişki araştırılmıştır.

**Gereç ve Yöntem:** 32. gebelik haftasından önce doğan ve solunum sıkıntısı nedeniyle entübe edilen bebeklerden yaşamın 1, 3, 14 ve 28. günlerinde bronkoalveolar lavaj sıvısı ve serum örnekleri alındı. BPD'li 21 yenidoğan ve BPD'siz 20 yenidoğan olmak üzere toplam 41 olgu çalışmaya dahil edildi.

**Bulgular:** CC16 düzeyleri BPD grubunda sürekli olarak daha yüksekti, 3. günde pik yaptı, 14. ve 28. günlerde anlamlı farklılıklar gösterdi ($p<0,05$). TGF-$\beta_1$, BPD grubunda 1. günde en yüksekti, 3. güne kadar azaldı, ancak kontrollerden daha yüksek kaldı ($p<0.05$). Kaspaz seviyeleri 1. günde benzerdi ancak 3. günde BPD grubunda önemli ölçüde daha yüksekti. 14. günde tüm kaspaz seviyeleri düşerken, 28. günde kaspaz-3 düşmeye devam etti ve kaspaz-8 ve -9 tekrar yükseldi. Özellikle ekstrensek yolak aracılığıyla artan kontrolsüz apoptoz, BPD gelişimiyle ilişkilendirilmiştir.

**Sonuç**: CC16, TGF-$\beta_1$ ve kaspazlar BPD için potansiyel erken belirteçlerdir ve yeni tedavi yöntemlerine rehberlik edebilir.

**Anahtar Kelimeler*:*** *Bronkopulmoner displazi, CC16, TGF-β1, kaspazlar*

## Introduction

Bronchopulmonary dysplasia (BPD) is a disease that occurs due to inflammation, fibrosis, developmental disorders, or lung tissue arrest in premature newborns receiving long-term high oxygen concentrations and high-pressure respiratory support (1, 2). Although its definition has changed over the years, its incidence has not decreased. It continues to be an important cause of mortality and morbidity in small preterm infants (3).

Clara cell secretory protein (CC16) is a protein secreted from non-ciliated Clara cells located throughout the entire tracheobronchial tree, especially in the terminal bronchioles, and has been shown to reduce surfactant degradation and oxidative stress (4). It is a potent immunosuppressive and anti-inflammatory agent (4-6).

* This study was summarized from Mehtap DURUKAN TOSUN's Specialization Thesis in Medicine.

Transforming growth factor beta (TGF-β) is a cytokine with inflammatory effects and has three isotypes. TGF-$\beta_1$ is involved in cellular development and maturation (7). An increase in TGF-$\beta_1$, which is considered a key cytokine in lung injury, plays a role in tissue fibrosis, inflammatory response regulation, and tissue healing. Studies have shown that it plays a role in tissue repair in animal models of lung injury. Excessive release during morphogenesis inhibits bronchial branching (7, 8)

Caspases are cysteine proteases. The proteins caspase-3 and caspase-9 play important roles in apoptosis. Its main function is to prevent DNA polymerase enzyme activity, which leads to cell apoptosis (9).

They are inactive in the cytoplasm and function by activating each other in a cascade (10). With the formation of the apoptosome, caspase-3 is activated, and the cell undergoes apoptosis (11).

This study aimed to investigate the relationships between CC16, TGF-$\beta_1$, caspase-3, caspase-8, and caspase-9 levels in serum and bronchoalveolar lavage fluid (BALF) samples and the early development of BPD in premature infants receiving ventilatory support.

## Materials and Methods

### Research and Publication Ethics:

Infants younger than 32 weeks of gestation who were hospitalized in the Neonatal Intensive Care Unit of Firat University, Pediatric Neonatal Intensive Care Unit, and Neonatal Intensive Care Unit of Elazig Training and Research Hospital between November 2009 and August 2010 were included in the study. Twenty-one infants who developed bronchopulmonary dysplasia were prospectively included in the study group, and 20 infants who did not develop BPD were included in the control group. Informed consent was obtained from the families. The samples were analyzed at Mersin University, Faculty of Pharmacy, Department of Biochemistry.

Demographic characteristics of the patients were recorded. Neonates with genetic diseases/malformations were not included in the study.

The diagnosis of bronchopulmonary dysplasia was determined by gestational age, oxygen demand at postnatal 28 and/or 36 weeks of gestation, and radiological findings (2).

Serum samples and BALF samples were obtained from the patients on postnatal days 1, 3, 14, and 28. Since the control group was not intubated for a long time, TGF-$\beta_1$ levels were studied in day 1-3 day samples, and caspase levels were studied only in day 3 samples

Caspase-3, Caspase-8, and Caspase-9 activity was evaluated quantitatively in cells obtained from bronchoalveolar lavage fluid by a colorimetric Protease Assay Kit. Caspases were it was measured with the Caspase-3, 8, 9/CPP32 Colorimetric Assay Kit (BioVision Research Products, USA) as described in the kit. The amount of pNA was determined by measuring the absorbance at 405 nm in a microtiter plate reader (ELX 800, Biotek, Türkiye).

Clara cell secretory protein and TGF-$\beta_1$ serum levels were determined by enzyme-linked immunosorbent assay (ELISA) using the sandwich principle with the Human Clara Cell Protein ELISA Kit (BioVendor Research and Diagnostic Products). The resulting color was detected and quantified at 450 nm using a microtiter plate reading device (ELX 800, Biotek, Türkiye).

The results were evaluated using the SPSS 16.0 (statistical package for social sciences) Windows statistical package program. The data are expressed as the mean ± standard deviation. The significance of the difference between the values of the patient and control groups was calculated using an independent-sample t-test. The variability of parameters at different time points within the same group was evaluated using paired samples t-tests. Independent sample two-way analysis of variance was used to examine the effect of time and disease factors. Values of $p<0.05$ were considered significant.

## Results

A total of 164 serum samples and 112 BALF samples from 41 premature infants born at or below 32 weeks of gestation were included in the study on the first, 3rd, 14th, and 28th days. There were 41 BALF samples collected on the first day and 3rd day. The number of samples decreased as the babies were extubated, and 17 samples were collected on day 14, while 10 BALF samples were collected on day 28. Twenty-one of the patients were diagnosed with BPD. Patients who died during the sampling period were excluded from the study. One of the patients who developed BPD died due to nosocomial sepsis during the period after specimen collection. The demographic characteristics of the patients are shown in Table 1.

There was no significant difference in the statistical CC16 level between the patient group and the control group on day 1, but there was a significant difference in the TGF-$\beta_1$ level ($p<0.05$). In the control group, caspase activity could not be evaluated because caspase activity was not measured on day 1 (Table 2).

On the 3rd day, there was no significant difference in CC16 expression between the patient group and the control group, whereas there was a significant difference in TGF-$\beta_1$, caspase-3, caspase-8, and caspase-9 expression ($p<0.05$) (Table 2).

There was a statistically significant difference between CC16 levels of the patient group and the control group on day 14 ($p<0.05$). In the control group, TGF-$\beta_1$ and caspase levels on day 14th could not be evaluated because they were not studied (Table 2).

On the 28th day, a statistically significant difference was found between CC16 levels in the patient group and

**Table 1.** Demographic characteristics of the patient group and control group-1

| | Patient group | Control group |
|---|---|---|
| Type of delivery (n, %) | | |
| Cesarean section | 13 (61.9) | 16 (80) |
| Vaginal | 8 (39.1) | 4 (20) |
| Gestation week (mean±sd) | 29.3±2.1 | 30.3±1.7 |
| (min-max) | (26-32) | (27-32) |
| Birth weight (g) (mean±sd) | 1368±395 | 1427±350 |
| (min-max) | (650-1950) | (900-2190) |
| Maternal age (mean±sd) | 27.8±4.9 | 27.8±4.9 |
| (min-max) | (20-39) | (20-39) |
| Invasive ventilation duration (hours) | 536±179.00 | 181.2±76.08 |
| (min-max) | (96-672) | (96-312) |
| Total oxygen time (hours) | 672±0.00 | 386.00±122.37 |
| (min-max) | (96-672) | (144-576) |
| Surfactant (n, %) | | |
| None | 2 (9.5) | 6 (30) |
| 1 dose | 11 (52.5) | 10 (50) |
| 2 dose | 6 (28.5) | 4 (20) |
| 3 dose | 2 (9.5) | - |
| Pneumothorax (n, %) | 2 (9.5) | - |

**Table 2.** 1$^{th}$, 3$^{rd}$, 14$^{th}$, 28$^{th}$ days CC16, TGF-$\beta_1$, caspase-3, caspase-8 and caspase-9 levels in the patients and control group

| | Patients Mean ± SD | Controls Mean ± SD | p |
|---|---|---|---|
| **1$^{st}$ day** | | | |
| CC16 (ng/mL) | 9.14±4.73 | 9.82±9.63 | 0.775 |
| TGF-$\beta_1$ (pg/mL) | 71.16±58.33 | 23.00±18.16 | <0.05 |
| Caspase-3* | 0.17±0.04 | | |
| Caspase-8* | 0.18±0.04 | | |
| Caspase-9* | 0.17±0.04 | | |
| **3$^{rd}$ day** | | | |
| CC16 (ng/mL) | 14.39±11.52 | 7.81±9.95 | 0.06 |
| TGF-$\beta_1$ (pg/mL) | 45.82±32.23 | 19.63±17.34 | <0.05 |
| Caspase-3 | 0.18±0.05 | 0.14±0.01 | <0.05 |
| Caspase-8 | 0.19±0.05 | 0.16±0.02 | <0.05 |
| Caspase-9 | 0.18±0.05 | 0.14±0.01 | <0.05 |
| **14$^{th}$ day** | | | |
| CC16 (ng/mL) | 9.22±4.63 | 5.84±3.49 | <0.05 |
| TGF-$\beta_1$ (pg/mL) | 36.76±24.98 | | |
| Caspase-3 | 0.17±0.02 | | |
| Caspase-8 | 0.18±0.03 | | |
| Caspase-9 | 0.16±0.02 | | |
| **28$^{th}$ day** | | | |
| CC16 (ng/mL) | 15.64±13.14 | 7.67±3.63 | <0.05 |
| TGF-$\beta_1$ (pg/mL) | 47.00±25.82 | | |
| Caspase-3 | 0.16±0.03 | | |
| Caspase-8 | 0.20±0.05 | | |
| Caspase-9 | 0.17±0.02 | | |

those in the control group ($p<0.05$). In the control group, evaluation could not be made because there was no TGF-$\beta_1$ or caspase activity on the 28th day (Table 2).

The variation in CC16 levels in the patient and control groups according to day is shown in Figure 1. Although the day 1 values were similar among the groups, an increase was observed in the patient group on day 3. However, this increase was not statistically significant. On the 14th day, a decrease in CC16 levels was observed in the patient and control groups. The difference was statistically significant. On the 28th day, there was a significant increase in both groups ($p<0.05$).

The variation in TGF-$\beta_1$ levels in the patient group and control group according to day is shown in Figure 1. The day 1 values of the patients were significantly greater than those of the control group. Although there was a decrease in the patient group on the 3rd day, the difference was statistically significant. The values on the 14th and 28th days could not be evaluated because they were not available in the control group.

The changes in caspase levels in the patient group are shown in Figure 2. On the 1st day, the caspase-3 and caspase-9 levels were equal, but the caspase-8 level was greater. Statistical evaluation could not be performed for the control group because caspase activity data were not available on day 1.

On the 3rd day, there was an increase in all 3 parameters, and caspase-3 and caspase-9 levels were found to be equal. Compared with that of the control group, the difference was statistically significant ($p<0.05$) (Figure 2).

When the 14th-day values were compared, a decrease was detected in all 3 parameters. Statistical evaluation could not be performed for the control group since caspase activity data were not available for day 14.

On the 28th day, caspase-8 and caspase-9 levels increased, and caspase-3 levels decreased compared to those on the 14th day. Statistical evaluation could not be performed for the control group since there were no caspase values on the 28th day. When the caspase results were evaluated collectively, it was observed that the increase in caspase levels on day 3 attracted increased attention, and apoptosis reached its maximum level on day 14. The extrinsic pathway was more dominant in triggering apoptosis.

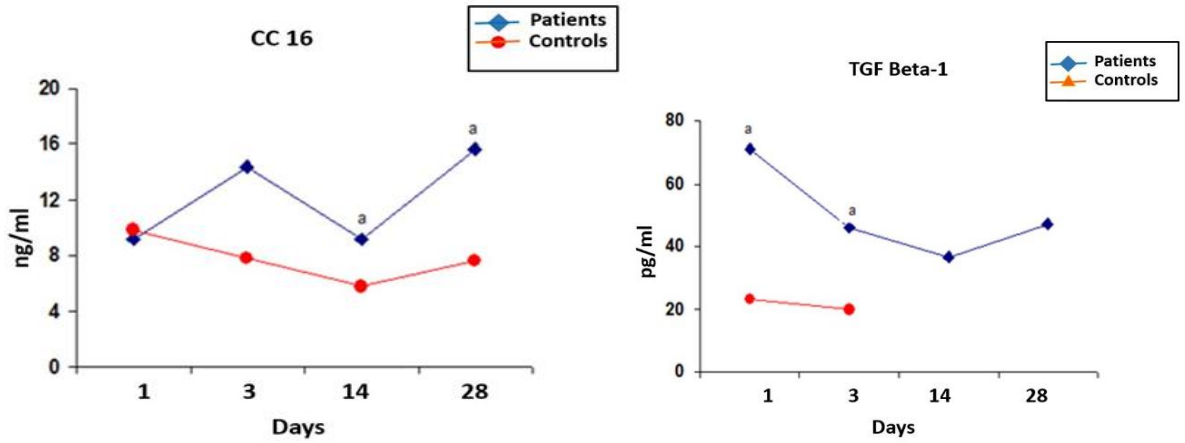


**Figure 1.** Variation of CC16 and TGF-$\beta_1$ levels according to day in both groups

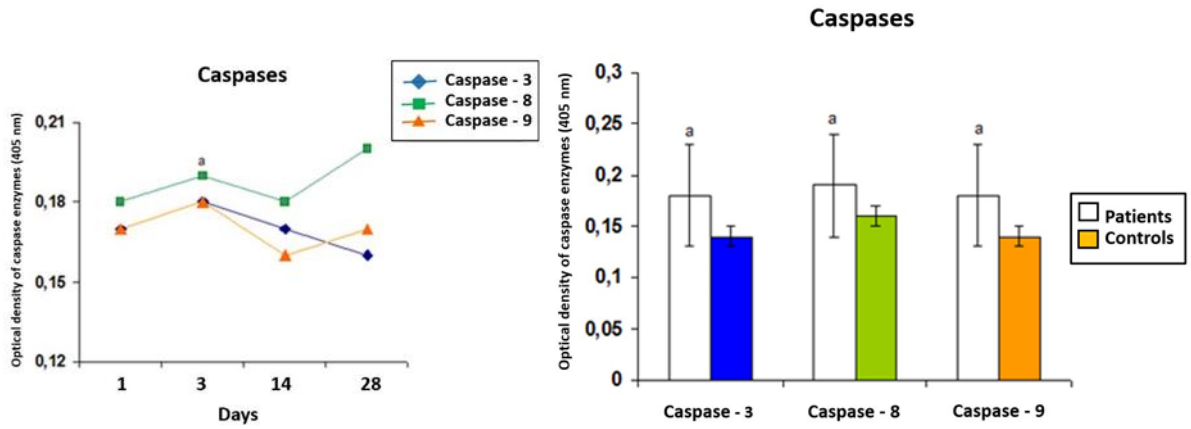


**Figure 2.** The first graph shows the change in caspases according to days in the patient group, and the second graph shows the change in caspases according to days in both groups

## Discussion

Bronchopulmonary dysplasia is the clinical condition with the highest mortality and morbidity among the long-term complications of prematurity. It is important because it causes frequent hospitalizations, growth retardation, and neuropsychomotor developmental delays (1, 2). In vitro studies have shown that CC16, a natural, potent immunosuppressive and anti-inflammatory agent, inhibits monocyte and polymorphic leukocyte chemotaxis and phagocytosis. It has many anti-inflammatory properties, such as inhibiting the release of substances that initiate inflammation and inhibit neutrophil activation (4).

As gestational age decreases, CC16 levels in serum and bronchoalveolar lavage fluid decrease, and there are ongoing studies on whether this predisposes individuals to the development of BPD (12).

Rallis et al. (4), examined the effect of perinatal factors on cord blood CC16 levels in a retrospective cohort study of 60 newborns born in 2023 with a gestational age (GA) <34 weeks. In neonates with a GA<32 weeks, cord blood CC16 concentrations were significantly lower in neonates with a GA between 320/7 and 33/6/7 weeks than in neonates with a GA between 32 and 33 weeks (7.6±2.9 ng/mL compared with 5.4±2.5, p=0.039). In this study, there was no significant difference between the groups in CC16 levels measured on day 1. On day 3, CC16 levels were increased but not significantly so in the BPD group, and CC16 levels were generally lower in the control group. Although this difference was not statistically significant, it may be explained by the fact that all of the patients had RDS and received mechanical ventilation support. In the literature, there are studies in which the CC16 level on the 1$^{st}$ postnatal day was found to be low, and there are also studies in which it was found to be high (13).

Sarafidis et al. (13), studied CC16 levels by taking serum samples in the first 2 hours after birth, at 72 hours, 14$^{th}$ day, and 36 weeks of gestation or at discharge in a study conducted with 35 neonates born at less than 31 weeks of gestation with or without ventilation support and found that CC16 levels were very low in the group without ventilation support compared to the group with ventilation support. Similarly, in the present study, the development of BPD and CC16 levels were significantly greater in the ventilated group with severe RDS.

In this study, the decrease observed in the patient group and the control group on day 14 may be explained by the improvement in RDS findings and the alleviation of inflammation. In addition, the increase in apoptosis induced by caspase-3 on day 14 and the decrease in the number of damaged cells may be the reasons for the low CC16 level. In patients with bronchopulmonary dysplasia, the increase in day 28 values may be explained by the chronic process of damage, alveolar damage caused by mechanical ventilation, and increased permeability of the bronchoalveolar-blood barrier. This hypothesis may explain the relationship with the development of bronchopulmonary dysplasia.

In 2017, Guzmán-Bárcenas et al. (14) examined CC16 levels in bronchoalveolar lavage fluid on days 1 and 7 in term and preterm infants intubated for respiratory distress and reported that CC16 levels were lower in the group that developed BPD than in the control group, and the CC16 levels measured on day 7 were lower than those measured on day 1. This result may lead to the hypothesis that 'The lack of a pulmonary protective effect due to low CC16 levels leads to the development of BPD'.

A study published by Jinle Lin et al. (15) in 2020 demonstrated the protective effect of rCC16 on lipopolysaccharide-induced pneumocyte apoptosis and the prevention of cellular damage. Rallis et al. (4) examined CC16 levels in cord blood and reported that CC16 levels were significantly lower in newborns with BPD than in those without BPD.

CC16 may be an important marker of pulmonary damage and the development of BPD.

TGF-$\beta_1$, which is involved in cellular proliferation and maturation, is a multifunctional protein (7). It regulates cell proliferation, differentiation and matrix production; embryonic development; wound healing; and pulmonary vascularization (16). Premature exposure to septation by disrupting normal molecular pathways. Importantly, TGF-β signaling is a key growth factor known to affect injury, mesenchymal homeostasis, and lung development (17-20). Although there are high levels of TGF-β in aspirates, the findings of animal studies linking altered TGF-β increases to alveolar development are contradictory. Chronic hyperoxia impairs septation and increased TGF-β results in alveolar disruption (21-25).

Kotecha et al. (26) measured activated and total TGF-$\beta_1$ levels in BALF in a study involving 15 patients diagnosed with RDS, 18 patients who developed BPD, and 7 patients who did not develop BDP and who were born at less than the 36$^{th}$ gestational week. TGF-$\beta_1$ levels were 30±21.1 ng/ml in infants with BPD, 6.5±2.4 ng/ml in patients with RDS, and 2.8±2.3 ng/ml in the control group. In the group with BPD, the increase was greatest on the 4$^{th}$ day. According to the results of Kotecha's study (26), TGF-$\beta_1$ can be used as an early marker in the development of BPD. In this study, TGF-$\beta_1$ levels on day 1 were significantly greater in the BPD group than in the control group. This result was consistent with the literature (22, 27).

Uncontrolled TGF release may predispose patients to lung tissue damage, the suppression of alveolarization, and the development of BPD. Gauldie et al. (22) first intranasally transferred the activated TGF-$\beta_1$ gene into adult rats via adenovirus, and it was histologically determined that progressive interstitial fibrosis developed in the adult rat lung within a few weeks after transfer. Then, the activated TGF-$\beta_1$ gene was transferred into one-day-old rats treated with the same vector via the same route, and BALF and lung tissue samples were collected for histological examination of 46 samples on days 3, 7, and 28. In the gene transfer group, an increase in TGF-$\beta_1$ levels was

observed in the BALF on all days, starting from day 3. Deterioration of the alveolar sac in the lung tissue on the 3rd day and alveolar septation, thick and hypercellular septae, and abnormal capillary development were observed on the 28th day. Considering the results of the study, it has been shown that uncontrolled release of TGF-$\beta_1$ causes severe pulmonary damage and leads to the progression of fibrosis. Similar to the results of Gauldie et al. (22), the TGF-$\beta_1$ levels in the patient group were significantly greater in the BD group than in the control group on day 3.

Jonsson et al. (25) measured TGF-$\beta_1$ levels in BALF in a study conducted in premature infants. In serial samples, TGF-$\beta_1$ levels were increased in patients without BPD. Similarly, in the present study, TGF-$\beta_1$ levels were increased on the 14th and 28th days in the BPD group. Similar to previous studies, it was thought that high levels of TGF-$\beta_1$, compared to those in the control group, may predispose individuals to the development of BPD by causing tissue damage.

In a study published in 2019 titled 'Mesenchymal stromal cells and TGF-$\beta_1$ in tracheal aspirate of premature infants: Early predictors for bronchopulmonary dysplasia?' (8) TGF-$\beta_1$ levels in tracheal aspirate samples obtained in the first week of life were high in patients who developed BPD, and it was concluded that TGF-$\beta_1$ could be used as an early marker. The relationship between the development of BPD and TGF-β has been established in studies conducted for many years (3).

All cells in the human body are in a constant state of construction and destruction. Apoptosis plays an important role in maintaining the natural balance and the continuation of cellular development and differentiation (25, 27). Caspase molecules are essential proteins that play a key role in the apoptotic process (25, 28-30).

Mokres et al. (31) evaluated angiogenesis, apoptosis via activated caspase-3, VEGF receptor-1 and receptor-2 levels, TGF-β activation, and elastin levels in the lung tissue of neonatal rats receiving oxygen and ventilation support. On the sixth day, the lung tissue was examined. Compared to those in the control group, in the lungs of rats receiving ventilation and oxygen support, a 3-fold increase in alveolar area, a 50% decrease in endothelial surface area and alveolar number, a 5-fold increase in apoptosis, a greater than 50% increase in lung elastin tissue, and a thickening of the alveolar wall structure were observed. In this study, there was no difference in caspase-3, 8, or 9 levels on day 1 in the BPD group. No comparison could be made in the control group since day 1 values were not analyzed. The reason for the lack of difference in the day 1 results may be that the immature lung is exposed to barotrauma and oxytrauma for a shorter period, and therefore, the cellular response is delayed.

The suppression of long noncoding RNA was experimentally shown to be protective against BPD in a study conducted by Tao et al. (32) using caspase 3 inhibition and miRNA 421. In this study, they prevented the development of BPD by inhibiting caspase-3.

Guthmann et al. (33) studied TNF-α, caspase-3, caspase-8, and caspase-9 in alveolar type II cells after 24 and 48 hours of $O_2$ exposure in rats. Immediately after birth, the groups given 100% $O_2$ for 24 and 48 hours were compared with the group breathing room air. Type II cells and alveolar macrophages were obtained from all 3 groups by bronchoalveolar lavage and grown in cell culture. TNF-α, caspase-3, and caspase-8 levels were increased in both groups exposed to hyperoxia compared to those in the normoxic group. No difference was observed in caspase-9 activity. After 24 and 48 hours of hyperoxia, caspase-3 and caspase-8 activities decreased in normoxic rats. There was no significant difference in caspase-9 levels. In this study, when the changes in caspase levels in the BPD group over time were analyzed, caspase-3, caspase-8, and caspase-9 levels were close to each other on the first day, whereas increases in the levels of all three caspases were observed on the 3rd day. On the 14th day, a decrease in the expression of all three caspases was observed. On the 28th day, caspase-3 levels continued to decrease, while caspase-8 and caspase-9 levels increased. Collectively, the caspase results revealed that apoptosis increased in the patient group, apoptosis was triggered on the 14th day, and the extrinsic pathway was more effective.

The limitation of this study may be the fewer patients and the fact that newborns born in a single province were not included in the study. Multicenter studies with a higher number of cases will be more decisive on the subject.

In this study, the relationships between bronchopulmonary dysplasia and CC16, TGF-$\beta_1$, caspase-3, caspase-8, caspase-9, and CC16 levels were found to be elevated at all stages of life, especially from the third day of life, in infants with BPD. Similarly, TGF-$\beta_1$ levels are elevated at all times in patients with BPD. Considering the results and the available literature, these two parameters may play important roles in the progression to BPD and may be important indicators for early diagnosis of the disease and determination of patient prognosis.

Caspase activity increased on the third day of life in patients with BPD and remained high in the following days, and the extrinsic pathway was more active, especially with caspase-8 activation. Caspase inhibitors, which are among the treatment examples developed by interfering with apoptosis, may be an alternative to medical treatment methods for BPD.

Many studies have been conducted to protect preterm infants from BPD, which is an important cause of mortality and morbidity. With the evaluation of the results obtained and the development of effective treatment methods, it will be possible to predict BPD, which is an important problem in premature infants, in the early period and to take important steps in the treatment and prevention of its complications.

## References


1. Northway Jr WH, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease: Bronchopulmonary dysplasia. New England Journal of Medicine 1967;276(7):357-368.
2. Bancalari E. Neonatal chronic lung disease. Neonatal-perinatal medicine. 2002:1057-1070.
3. Gilfillan M, Bhandari A, Bhandari V. Diagnosis and management of bronchopulmonary dysplasia. BMJ 2021;375.
4. Rallis D, Papathanasiou AE, Christou H. Pneumoprotein CC16 in the umbilical cord blood of preterm neonates. American Journal of Perinatology 2024;41(S 01):e3367-e3373.
5. Martinu T, Todd JL, Gelman AE, Guerra S, Palmer SM. Club cell secretory protein in lung disease: Emerging concepts and potential therapeutics. Annual Review of Medicine 2023;74(1):427-441.
6. Laing IA. CC16: A Biomarker of pollutant exposure and future lung disease? American Journal of Respiratory and Critical Care Medicine 2019;200(5), 529-530.
7. Ruschkowski BA, Esmaeil Y, Daniel K, et al. Thrombospondin-1 plays a major pathogenic role in experimental and human bronchopulmonary dysplasia. American Journal of Respiratory and Critical Care Medicine 2022;205(6):685-699.
8. Aly H, Mansi Y, Ez El Din Z, Gabr Metwally H, Sabry A. Mesenchymal stromal cells and TGF-$\beta_1$ in tracheal aspirate of premature infants: early predictors for bronchopulmonary dysplasia? Journal of Perinatal Medicine 2019;47(4):470-477.
9. Aslan A, Beyaz S, Gok O, Erman O. The effect of ellagic acid on caspase-3/bcl-2/Nrf-2/NF-kB/TNF-α/COX-2 gene expression product apoptosis pathway: A new approach for muscle damage therapy. Molecular Biology Reports 2020;47:2573-2582.
10. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell Death & Differentiation 2015;22(4):526-539.
11. Abedin M, Wang D, McDonnell M, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. Cell Death & Differentiation 2007;14(3):500-510.
12. Rallis D, Baltogianni M, Dermitzaki N, et al. Club cell protein expression amongst infants with respiratory distress syndrome. Pediatric Pulmonology 2022;57(6):1543-1546.
13. Sarafidis K, Stathopoulou T, Diamanti E, et al. Clara cell secretory protein (CC16) as a peripheral blood biomarker of lung injury in ventilated preterm neonates. European Journal of Pediatrics 2008;167:1297-1303.
14. Guzmán-Bárcenas J, Calderón-Moore A, Baptista-González H, Irles C. Clara cell protein expression in mechanically ventilated term and preterm infants with respiratory distress syndrome and at risk of bronchopulmonary dysplasia: A pilot study. Canadian Respiratory Journal 2017;2017(1):8074678.
15. Lin J, Li J, Shu M, et al. The rCC16 protein protects against LPS-induced cell apoptosis and inflammatory responses in human lung pneumocytes. Frontiers in Pharmacology 2020;11:1060.
16. Li R. Disrupted TGF-β signaling: A link between bronchopulmonary dysplasia and alveolar type 1 cells. The Journal of Clinical Investigation 2024;134(6).
17. McGowan SE. Paracrine cellular and extracellular matrix interactions with mesenchymal progenitors during pulmonary alveolar septation. Birth Defects Research Part A: Clinical and Molecular Teratology 2014;100(3):227-239.
18. Ahlfeld SK, Conway SJ. Assessment of inhibited alveolar-capillary membrane structural development and function in bronchopulmonary dysplasia. Birth Defects Research Part A: Clinical and Molecular Teratology 2014;100(3):168-179.
19. Jobe AH. The new bronchopulmonary dysplasia. Current Opinion in Pediatrics 2011;23(2):167-172.
20. Abman SH, Conway SJ. Developmental determinants and changing patterns of respiratory outcomes after preterm birth. Wiley Online Library; 2014;100(3):127-133.
21. Sureshbabu A, Syed MA, Boddupalli CS, et al. Conditional overexpression of TGFβ1 promotes pulmonary inflammation, apoptosis and mortality via TGFβR2 in the developing mouse lung. Respiratory Research. 2015;16:1-12.
22. Gauldie J, Galt T, Bonniaud P, et al. Transfer of the active form of transforming growth factor-β1 gene to newborn rat lung induces changes consistent with bronchopulmonary dysplasia. The American Journal of Pathology 2003;163(6):2575-2584.
23. Alejandre-Alcázar MA, Kwapiszewska G, Reiss I, et al. Hyperoxia modulates TGF-β/BMP signaling in a mouse model of bronchopulmonary dysplasia. American Journal of Physiology-Lung Cellular and Molecular Physiology 2007;292(2):L537-L49.
24. Ahlfeld SK, Wang J, Gao Y, Snider P, Conway SJ. Initial suppression of transforming growth factor-β signaling and loss of TGFBI causes early alveolar structural defects resulting in bronchopulmonary dysplasia. The American Journal of Pathology 2016;186(4):777-793.
25. Jonsson B, Li YH, Noack G, Brauner A, Tullus K. Downregulatory cytokines in tracheobronchial aspirate fluid from infants with chronic lung disease of prematurity. Acta Paediatrica 2000;89(11):1375-1380.
26. Kotecha S, Wangoo A, Silverman M, Shaw R. Increase in the concentration of transforming growth factor beta-1 in bronchoalveolar lavage fluid before development of chronic lung disease of prematurity. The Journal of Pediatrics. 1996;128(4):464-469.
27. Liu M, Iosef C, Rao S, et al. Transforming growth factor–induced protein promotes NF-κB–mediated angiogenesis during postnatal lung development. American Journal of Respiratory Cell and Molecular Biology 2021;64(3):318-330.
28. Raff MC. Social controls on cell survival and cell death. Nature 1992;356(6368):397-400.
29. Ellis RE, Yuan J, Horvitz HR. Mechanisms and functions of cell death. Annual Review of Cell Biology 1991;7(1):663-698.

30. Asadi M, Taghizadeh S, Kaviani E, et al. Caspase-3: structure, function, and biotechnological aspects. Biotechnology and Applied Biochemistry 2022;69(4):1633-1645.

31. Mokres LM, Parai K, Hilgendorff A, et al. Prolonged mechanical ventilation with air induces apoptosis and causes failure of alveolar septation and angiogenesis in lungs of newborn mice. American Journal of Physiology-Lung Cellular and Molecular Physiology 2010;298(1):L23-L35.

32. Tao X, Fang Y, Huo C. Long non-coding RNA Rian protects against experimental bronchopulmonary dysplasia by sponging miR-421. Experimental and Therapeutic Medicine 2021;22(1):1-10.

33. Guthmann F, Wissel H, Schachtrup C, et al. Inhibition of TNFalpha in vivo prevents hyperoxia-mediated activation of caspase 3 in type II cells. Respiratory Research 2005;6:1-16.