The Turkish Journal of Pediatrics 2017; 59: 122-129
DOI: 10.24953/turkjped.2017.02.003

Original

Low serum IGF-1 and increased cytokine levels in tracheal aspirate samples are associated with bronchopulmonary dysplasia

Cansu Yılmaz[1], Nilgün Köksal[2], Hilal Özkan[2], Bayram Ali Dorum[2], Onur Bağcı[2]

[2]Division of Neonatology, [1]Department of Pediatrics, Uludağ University Faculty of Medicine, Bursa, Turkey.
E-mail: bayramalidorum@gmail.com

Received: 22nd November 2016, Revised: 15th February 2017, Accepted: 20th February 2017

SUMMARY: Yılmaz C, Köksal N, Özkan H, Dorum BA, Bağcı O. Low serum IGF-1 and increased cytokine levels in tracheal aspirate samples are associated with bronchopulmonary dysplasia. Turk J Pediatr 2017; 59: 122-129.

Despite developments in the perinatal and neonatal care, bronchopulmonary dysplasia (BPD) is still the most frequently seen long-term complication in preterm infants. The aim of this prospective study is to investigate the association between the development of BPD and serial measurements of IGF-1 levels and their relationship with levels of IGF-1 and cytokine in tracheal aspirate fluids. A total of 40 premature infants, born at a gestational age of ≤ 32 weeks, were enrolled in the study. On postnatal day-1, 3, 7, 21 and 28 serum IGF-1 levels and IGF-1 levels, IL-6, IL-8, IL-10 and TNF-alpha levels in tracheal aspirate fluid samples of intubated cases were examined. Mean gestational age of 40 patients included in the study was 29.41 ± 2.23 weeks, and their mean birth weight was 1,256.85 ± 311.48 g. BPD was detected in 35% of cases. Mean gestational week and birth weight of the cases that developed BPD were 30 ± 3 weeks and 1,150 ± 295 g, respectively. Serum IGF-1 levels on postnatal day-1, 3, 7, 21 and 28 in cases who developed BPD were significantly lower when compared with those without BPD ($p < 0.01$). Levels of IL-6, IL-8, IL-10, and TNF-alpha in tracheal aspirate samples were significantly higher in cases with BPD compared to those without BPD ($p<0.05$). IGF-1 levels in tracheal aspirate fluid samples did not differ significantly based on the presence of BPD ($p>0.05$). Severity of BPD was associated with decreased serum IGF-1 levels and increased cytokine levels in tracheal aspirate samples.

Key words: bronchopulmonary dysplasia, cytokines, IGF-1, premature, tracheal aspirate fluid.

Bronchopulmonary dysplasia (BPD) is one of the most frequent causes of long-term morbidities in preterm infants[1]. In addition to respiratory problems, BPD can also lead to neurodevelopmental retardation. Many factors including prematurity, mechanical ventilation, oxygen therapy or genetic factors play a role in the pathogenesis of BPD[2]. Among these factors antenatal infection and inflammation also play an important role. During the inflammatory process, which emerges as a result of different causes as prenatal, postnatal infection, mechanical ventilation, hypoxic injury, and hyperoxic damage many pro-inflammatory and anti-inflammatory cytokines play a role. Some studies have investigated the correlation between BPD and pro-inflammatory cytokines as interleukin (IL)-1, 6, 8, TNF-α, and anti-inflammatory cytokines as IL-10[3,4].

Some studies have also demonstrated the potentially significant role of IGF-1, which involves pulmonary alveolarization and healing of tissue injuries, in the development of BPD[5,6].

In this study, we aimed to reveal the correlations between the development of BPD in premature infants, IGF-1 levels in serum and tracheal aspirate (TA) and cytokine levels in TA measured on separate days during the postnatal period of these infants.

Material and Methods

This prospective study was conducted in the Neonatal Intensive Care Unit (NICU) of Uludag University Faculty of Medicine, between January 2015 and December 2015. Approval of the Ethics Committee of Uludag University Faculty of Medicine was obtained on 01.28.2014 (decision # 2014-2/24). Written and undersigned, enlightened and completed consent forms were obtained from all parents/guardians of all infants.

A total of 40 premature infants born at a gestational age of ≤ 32 weeks were enrolled in this prospective study. Infants with major congenital anomalies and infants exited during the study period were excluded from the study. Demographic data and follow-up characteristics of the patients were recorded.

Venous blood samples were drawn from all cases on postnatal day-1, 3, 7, 21 and 28 and centrifuged. Serum portions of the blood samples were transferred into Eppendorf tubes and stored at -20°C.

Tracheal aspirate samples were collected during routine endotracheal suction on the first day of life. For the suctioning procedure, the infant was positioned in a supine posture with the head in the midline, then, 1 ml of saline was instilled into the endotracheal tube, after that the ventilator was reconnected for three to four breaths, and the endotracheal tube was suctioned once or twice with a suction catheter (6 French gauge). The catheter was rinsed with 2 ml of saline, and the aspirate was collected into a sterile trap. The samples were centrifuged at 3,000 rpm for 5 minutes

Table I. Demographic Features of Patients According to the Development of Bronchopulmonary Dysplasia.

Features	Bronchopulmonary Dysplasia		p
	Present (N=14)	Absent (N=26)	
Birth weight (gram)	1150 (630-1610)	1400 (715-2005)	[b]0.015*
Gestational age (weeks)	24-32 (28.14)	27-32 (31.29)	[b]0.002**
Female gender	7 (50.0)	14 (53.8)	[b]1.000
Cesarean section	13 (92.9)	22 (84.6)	[c]0.640
Early membrane rupture	3 (21.4)	6 (23.1)	[c]1.000
Chorioamnionitis	2 (14.3)	1 (3.8)	[c]0.276
Maternal diabetes	1 (7.1)	0 (0.0)	[c]0.350
Intrauterine growth retardation	2 (14.3)	6 (23.1)	[c]0.689
Preeclampsia	4 (28.6)	8 (30.8)	[c]1.000
Antenatal steroid administration	12 (85.7)	19 (73.1)	[d]0.674
Apgar 1st minute	4 (0-8)	5 (0-9)	[a]0.069
Apgar 5th minute	6 (3-9)	7 (2-10)	[a]0.055
Duration of O_2 treatment (day)	43 (13-208)	17.5 (2-178)	[a]0.001**
Duration of invasive MV (day)	16.5 (0-115)	2 (0-92)	[a]0.003**
Duration of non-invasive MV (day)	18 (1-93)	7 (1-70)	[a]0.121
Duration of total parenteral nutrition	22 (11-60)	13 (7-80)	[a]0.033*
Surfactant replacement therapy	12 (85.7)	15 (57.7)	[d]0.213
Respiratory distress syndrome	11 (78.6)	14 (53.8)	[b]0.712
Retinopathy of prematurity	6 (42.9)	5 (19.2)	[d]0.177
Necrotizing enterocolitis	1 (7.1)	5 (19.2)	[d]1.000
Intraventricular hemorrhage (Stage III-IV)	1 (7.1)	3 (11.5)	[d]0.847
Sepsis	7 (50.0)	10 (38.5)	[b]0.712
Patent ductus arteriosus	10 (71.4)	9 (34.6)	[b]0.059

Data is presented as median (minimum-maximum) or n (%) as appropriate. MV: mechanical ventilation.
[a]: Mann: Whitney U test; [b]: Yates continuity correction test; [c]: Fisher's exact test; [d]: Fisher Freeman Halton test

Table II. Serum IGF-1 Levels According to the Development of Bronchopulmonary Dysplasia.

Parameters	Bronchopulmonary Dysplasia		
	Present (N=14)	Absent (N=26)	[a]p
Serum IGF-1 on day-1 (ng/ml)	13.76 (8.92-13.76)	31.17 (9.73-45.67)	<0.001
Serum IGF-1 on day-3 (ng/ml)	12.01 (4.24-28.26)	29.85 (4.75-44.46)	<0.001
Serum IGF-1 on day-7 (ng/ml)	13.11 (3.75-29.26)	33.76 (9.06-42.86)	<0.001
Serum IGF-1 on day-21 (ng/ml)	12.72 (7.82-27.84)	29.28 (10.64-43.22)	<0.001
Serum IGF-1 on day-28 (ng/ml)	15.5 (4.24-31.68)	40.4 (9.65-50.12)	<0.001

Data is presented as median (minimum-maximum). IGF-1: insulin-like growth factor-1.
[a]Mann Whitney U Test

to separate supernatant and cellular fractions. Samples were frozen within ~1 hour of collection and stored at −20°C prior to analysis.

In the Immunology Laboratory of Uludağ University Faculty of Medicine IGF-1, TNF-α, IL-6, IL-8 and IL-10 levels in tracheal aspirates were measured. IGF-1 levels in serum samples collected on day-1, 3, 7, 21, and 28 were analyzed using commercial ELISA kits.

Diagnosis of BPD was made based on the currently accepted definition by USA National Institutes of Health[1]. Based on oxygen dependency the patients were classified as mild, moderate and severe BPD.

Retinal examinations were performed in consideration of screening and follow-up criteria of retinopathy of premature infants (ROP) stated in the guidelines of American Academy of Pediatrics[7]. Diagnosis of necrotizing enterocolitis (NEC) was determined based on Bell classification[8]. Pediatric cardiologist made the diagnosis of patent ductus arteriosus based on Doppler echocardiographic findings. Intraventricular bleeding (IVH) was detected on transfontanelle ultrasonography (TFUS) and classified based on Papile classification[9]. Patients with symptoms of sepsis whose blood cultures demonstrated bacterial growth were evaluated as sepsis.

Statistical analysis

For statistical analysis, SPSS 23.0 for Windows (SPSS, Chicago, IL, USA) program was used. When evaluating study data, in addition to descriptive statistical methods (mean, standard deviation, median, frequency, rate, minimum, maximum) fitness of data to a normal distribution was determined using Kolmogorov-Smirnov test. Intergroup comparisons of variables with non-normal distribution Mann-Whitney U test was used. For comparison of qualitative data Fisher-Freeman-Halton, Fisher's exact test and Yates' continuity correction test (chi-square test with Yates correction) were used. In the determination of cut-off values for variables, diagnosis screening tests (sensitivity, specificity, PPV, NPV) and ROC Curve analysis were used. Significance was evaluated at $p<0.05$.

Results

A total of 40 infants [21 female (52.5%) and 19 male (47.5%)] hospitalized in the NICU of

Table III. Serum IGF-1 Levels According to Severity of Bronchopulmonary Dysplasia.

Parameters	Severity of bronchopulmonary dysplasia		[a]p
	Mild to moderate (N=8)	Severe (N=6)	
Serum IGF-1 on day-1 (ng/ml)	19.18 (15.60-27.82)	10.76 (8.92-16.17)	0.001
Serum IGF-1 on day-3 (ng/ml)	21.74 (15.77-28.26)	9.80 (4.24-16.12)	<0.001
Serum IGF-1 on day-7 (ng/ml)	22.90 (16.35-29.26)	11.14 (3.75-18.31)	<0.001
Serum IGF-1 on day-21 (ng/ml)	19.99 (16.40-27.84)	11.85 (7.82-16.59)	0.01
Serum IGF-1 on day-28 (ng/ml)	22.90 (17.22-31.68)	14.09 (4.24-18.14)	0.02

Data is presented as median (minimum-maximum). IGF-1: Insulin-like growth factor-1.
[a]: Mann Whitney U Test

Table IV. Cut-off Values for Serum IGF-1 Levels as a Parameter in the Prediction of Bronchopulmonary Dysplasia (ROC Analyses).

Days	Cut-off value (ng/ml)	ROC curve area (%95 CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Day-1	≤16.17	0.874 (0.73-0.96)	100.00	80.77	73.70	100.00
Day-3	≤16.12	0.874 (0.73-0.96)	100.00	80.77	73.70	100.00
Day-7	≤18.31	0.874 (0.73-0.96)	100.00	80.77	73.70	100.00
Day-21	≤16.59	0.874 (0.73-0.96)	100.00	80.77	73.70	100.00
Day-28	≤18.14	0.874 (0.73-0.96)	100.00	80.77	73.70	100.00

NPV: negative predictive value; PPV: positive predictive value; ROC: Receiver operating characteristic

Uludağ University Faculty of Medicine, between January 1st and December 31st, 2015 were included in the study.

BPD was detected in 35% (N=14) of the cases. Mild (N=5; 36%), moderate (N=3; 21%) and severe (N=6; 43%) BPD developed in those cases. Based on the development of BPD, the patients were divided into 2 groups and their demographic and clinical characteristics were compared. Accordingly, a difference was not observed between types of delivery and gender of the patients in both groups, while birthweights in patients who developed BPD were significantly lower when compared with the other group (1,150 ± 295 g *vs* 1,392 ± 286 g) and gestational ages (28.14 ± 2.1 weeks *vs* 31.29±1.6 weeks) (Table I).

Serum IGF-1 values of the patients who developed BPD were found to be significantly lower (Table II). When patients with diagnosis of severe BPD were compared with those diagnosed as mild to moderate BPD, IGF-1 levels in the group who developed severe BPD had significantly lower IGF-1 levels (Table III).

ROC analyses were performed to evaluate usability of low serum IGF-1 level as a parameter in the prediction of BPD. Therefore, cut-off values for serum IGF-1 for postnatal day-1 (16.17 ng/ml), day-3 (16.12 ng/ml), day-7 (18.31 ng/ml), day-21 (16.59) and day-28 (18.14 ng/ml) were determined as indicated. Corresponding measurements below these levels were detected to have 80% specificity and 100% sensitivity (Table IV).

Thirty-two patients (total N=32) out of who completed the study were intubated and samples of TA were obtained from these 32 patients. Thirteen of these 32 patients developed BPD and the remaining 19 patients did not. Levels of IGF-1, IL-6, IL-8, IL-10 and TNF-alfa in TA samples of the patients in both groups were analyzed. Any intergroup difference between IGF-1 levels was not detected in TA samples of the patients who developed BPD, while cytokine levels in TA samples of the patients in whom BPD developed were significantly higher (Table V). In Table VI, groups were compared according to severity of BPD.

Discussion

In line with evolving neonatal care in recent years, survival rates of very low birth infants have increased. Current studies have been performed to decrease morbidities of these surviving infants. BPD leads the way among these relevant issues[1].

The incidence of BPD increases inversely with gestational age. In our center 276 premature infants born at 24-36 gestational ages in the year 2006 were investigated and BPD was detected in 30% of these infants[10]. Nievas et al.[11] reported that incidence of BPD increased up to 70 percent. In the present study, we examined premature infants born at a gestational age of ≤ 32 weeks and BPD developed in 14 of 40 (35%) infants included in the study.

Risk factors of BPD have been investigated in many studies and data indicating association between BPD and prematurity, inflammation, oxygen therapy, surfactant deficiency, PDA, barotrauma, volutrauma, maternal preeclampsia, and chorioamnionitis have been detected[10,12].

Maternal chorioamnionitis and premature membrane rupture have been thought to lead to intrauterine inflammation with resultant development of BPD. The study by Ozkan

Table V. Levels of IGF-1, IL-6, IL-8, IL-10 and TNF-alpha in Tracheal Aspirate Samples According to the Development of Bronchopulmonary Dysplasia on the First Day.

Parameters in tracheal aspirate	Bronchopulmonary Dysplasia		[a]p
	Present (N=13)	Absent (N=19)	
IGF-1 (ng/ml)	18.31 (0-21.31)	16.38 (0-21.19)	0.066
IL-6 (pg/ml)	0.98 (0-201.73)	0.10 (0-0.94)	<0.001
IL-8 (pg/ml)	1,617.96 (0-2,413.46)	0.36 (0-1,337)	<0.001
IL-10 (pg/ml)	2.87 (0-123.4)	0.02 (0-2.48)	<0.001
TNF-alfa (pg/ml)	0.53 (0-27.93)	0.34 (0-0.53)	<0.001

Data is presented as median (minimum-maximum). IGF-1: Insulin-like growth factor 1; IL: interleukin.
[a]: Mann Whitney U Test

et al.[10] also supports these assumptions. However, data obtained from our study do not support the presence of a significant correlation between development of BPD, EMR, and chorioamnionitis.

Similarly, neonatal sepsis can presumably lead to development of BPD[2]. In our study, a significant difference could not be found as for sepsis between infants who developed and did not develop BPD.

Maternal preeclampsia has been shown to increase frequency and severity of BPD[13]. In our study preeclampsia was detected in comparable number of infants who developed and did not develop eclampsia.

Besides, antenatal steroid use did not differ significantly between both groups as for gestational diabetes and IUGR and 1st and 5th minute Apgar scores of both groups were also comparable.

In our study, the number of days under mechanical ventilation, oxygen therapy and TPN of intubated cases with BPD were found to be significantly higher when compared with those in whom BPD did not develop ($p < 0.05$). Mechanical ventilation plays an important role in the development of BPD by inducing barotrauma and inflammation in developing alveoli and risk of BPD increases with increasing mechanical ventilation time[1,14,15]. A significant correlation was not detected between noninvasive mechanical ventilation times and development of BPD.

Various factors have been implicated in the pathogenesis of BPD including exposure to mechanical ventilation, increased levels of free oxygen radicals, immature antioxidant system, maternal chorioamnionitis, postnatal infections, inflammation caused by pro-inflammatory cytokines released from neutrophils and macrophages, symptomatic PDA, deficient postnatal protein intake and genetic predisposition predominantly prematurity and pulmonary immaturity.

In a study by Ohkawa et al.[5] the authors demonstrated that lower levels of IGF-1 at birth increased stepwise during the first 8 weeks of life. However, Löfqvist et al.[6] displayed the association between decrease in IGF-I levels during early postnatal period and development of BPD. Serum IGF-1 levels increase in line with gestational age, however in infants who developed BPD its levels do not increase. Also, in infants who developed BPD, serum IGF-1 levels were statistically significantly lower when compared with those without BPD A significant increase was not observed in IGF-1 levels in line with increasing age.

IGF-1 has been thought to play a role in the development of BPD by effecting alveolar angiogenesis especially through VEGF signalization[16]. Hellström et al.[17] detected lower IGF-1 levels in premature infants in whom comorbidities as ROP, IVH and NEC had developed. In another study, the usability of lower IGF-1 levels as a predictive parameter for the development of BPD was investigated. Low specificity and sensitivity of IGF-1 levels of <9.67 ng/ml, on 1st and < 7.26 ng/ml on 7th postnatal days in the prediction of BPD were determined[18]. However, this study has demonstrated that lower IGF-1 levels could be used in the prediction of BDP with higher sensitivity. Therefore, predictive cut-off values were determined for postnatal on day-1, 3,

Table VI. Levels of IGF-1, IL-6, IL-8, IL-10 and TNF-alpha in Tracheal Aspirate Samples According to the Severity of Bronchopulmonary dysplasia on the First Day.

Parameters in tracheal aspirate	Bronchopulmonary Dysplasia		
	Mild to moderate (N=8)	Severe (N=6)	[a]p
IGF-1 (ng/ml)	19.28 (16.82-21.31)	19.16 (15.52-21.12)	0.587
IL-6 (pg/ml)	0.88 (0.67-0.98)	26.11 (10.33-59.63)	0.001
IL-8 (pg/ml)	1,197 (227.53-1617.96)	1,775.55 (1723.5-1,994.97)	0.002
IL-10 (pg/ml)	1.70 (0.29-2.87)	18.67 (3.26-47.76)	0.007
TNF-alfa (pg/ml)	0.51 (0.47-0.53)	0.58 (0.54-0.63)	<0.001

Data is presented as median (minimum-maximum). IGF-1: Insulin-like growth factor 1, IL: interleukin.
[a]: Mann Whitney U Test

7, 21, and 28 as 16.17 ng/ml, 16.12 ng/ml, 18.31 ng/ml, 16.59 ng/ml and 18.14 ng/ml, respectively.

Capoluongo et al.[19] demonstrated increased levels of free IGF-1 levels in TA fluid samples collected at early phase of development of BPD in premature infants. However, in our study, any correlation could not be found between IGF-1 levels in TA samples of intubated infants and development of BPD.

The increase in concentrations of inflammatory cytokines in TA samples is associated with pulmonary inflammation. Major pro-inflammatory cytokines playing a role in this process progressing to BPD are IL-6, 8 and TNF-alpha. The relationship between anti-inflammatory cytokine IL-10 and development of BPD has been also investigated in various studies[4].

Increased concentrations of pro-inflammatory cytokine IL-6 have been demonstrated in TA samples collected on the first day of their lives of infants with RDS and BPD[20]. Bagchi et al.[21] reported significantly higher levels of IL-6 activity in pulmonary lavage fluids of infants who developed BPD which were maintained for the first 2 weeks of their lives and IL-6 levels started to drop after postnatal 28th days. Similarly, correlations between increased IL-6 concentrations in TA samples and duration of mechanical ventilation and higher IL-6 levels in infants who developed BPD have been demonstrated[22]. Tullus et al.[11] evaluated correlations between levels of various pro-inflammatory cytokines in TA samples of ventilated premature infants and development of BPD and detected significantly higher levels of IL-6 concentrations in TA samples in infants who developed BPD. In compliance with literature findings we also detected significantly higher levels of IL-6 in TA samples of infants who developed BPD.

Kotecha et al.[23] detected higher IL-8 concentrations in TA samples in infants who developed BPD within the first 10 days of their lives. In cases with severe disease, IL-8 levels increase in pulmonary injury, however this condition is not specific for BPD. In a study by Shimotake et al.[24] higher IL-8 levels in TA samples stimulated by NF-kB were detected in ventilated premature infants. Also in our study, we detected higher IL-8 levels in TA samples of premature infants collected at first postnatal day of premature infants.

TNF-α is one of the important components of cytokine-mediated host defense against bacteria, mycobacteria, fungi and parasites and various studies have suggested its potential association with BPD[25]. In a study performed, higher TNF-α levels in TA samples obtained within 2 hours after birth had been associated with worse pulmonary outcomes[26]. In another study, correlations between increased TNF-α levels in TA samples and duration of mechanical ventilation were demonstrated in infants who developed BPD[22]. In our study, TNF-α concentrations in TA samples collected within the first postnatal 24 hours were significantly higher in infants who developed BPD when compared with the infants who did not.

In the literature, controversial outcomes have been reported concerning the role of IL-10

impact of the development of BPD in preterm infants. Some studies reported lower IL-10 levels in both blood and TA samples that were obtained on the first day of life, some reported higher levels on the first day of life[20,27,28,30,31]. Schneibel et al.[3] have found that IL-10 levels of infants who developed and did not develop BPD were similar. Similarly, in a separate study IL-10 was detected in TA samples of the ventilated premature infants during the early stage of BPD, though its correlation with BPD has not been fully revealed[32]. Köksal et al.[4] reported that they have found significantly lower IL-10 levels in both serum and TA sample of premature infants who subsequently developed BPD. Conversely, in our study IL-10 levels in TA samples of the premature infants who developed BPD were higher relative to those who did not. Because of all this, the serial measurement of IL-10 levels, in a large sample of preterm infants after birth, are warranted in order to elucidate the association between development of BPD and IL-10 levels[4].

As a result of this study, significantly lower serum IGF-1 levels were detected in babies who developed BPD relative to those who did not. Besides levels of cytokines as IL-6, IL-8, IL-10, TNF-alpha in TAs obtained from intubated infants were significantly higher in infants who developed BPD when compared with those who did not. An association between IGF-1 levels in TA samples of intubated infants and development of BPD was not detected. Besides in our study, it has been determined that lower serum IGF-1 levels can be used in the prediction of BPD with higher sensitivity and thus relevant cut-off values were specified.

It has been conceived that precise determination of the roles of various cytokines in the development of the lungs and pulmonary injury, subsequently specification of treatment strategies to be developed which will target cytokines and/or their receptors will be beneficial in preventing BPD and/or related deaths in premature infants. To determine the correlation between BPD and serum IGF-1 levels and its potential use as an early marker for BPD; further randomized controlled studies are required.

Limitations of our study include limited number of cases and failure to analyze serum cytokine levels. It has been thought that differences in study outcomes cited in the literature may stem from timing of sample collection and characteristics of the study groups.

REFERENCES

1. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001; 163: 1723-1729.
2. Bancalari E. Epidemiology and risk factors for the "new" bronchopulmonary dysplasia. Neoreviews 2000; 1: e2-e5.
3. Schneibel KR, Fitzpatrick AM, Ping X-D, Brown LAS, Gauthier TW. Inflammatory mediator patterns in TA and their association with bronchopulmonary dysplasia in very low birth weight neonates. J Perinatol 2013; 33: 383-387.
4. Köksal N, Kayik B, Çetinkaya M, et al. Value of serum and bronchoalveolar fluid lavage pro- and anti-inflammatory cytokine levels for predicting bronchopulmonary dysplasia in premature infants. Eur Cytokine Netw 2012; 23: 29-35.
5. Ohkawa N, Shoji H, Kitamura T, et al. IGF-I, leptin and active ghrelin levels in very low birth weight infants during the first 8 weeks of life. Acta Paediatr 2010; 99: 37-41.
6. Löfqvist C, Hellgren G, Niklasson A, Engström E, Ley D, Hansen-Pupp I. Low postnatal serum IGF-I levels are associated with bronchopulmonary dysplasia (BPD). Acta Paediatr Int J Paediatr 2012; 101: 1211-1216.
7. Academy A, Ophthalmology P. Screening examination of premature infants for retinopathy of prematurity. Ophthalmology 1997; 104: 888-889.
8. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33: 179-201.
9. Papile LA, Munsick-Bruno G, Schaefer A. Relationship of cerebral intraventricular hemorrhage and early childhood neurologic handicaps. J Pediatr 1983; 103: 273-277.
10. Özkan H, Köksal N, Çetinkaya M, Canıtez Y. Bronkopulmoner displazide risk faktörleri. Güncel Pediatri 2008; 6: 66-71.
11. Nievas FF, Chernick V. Bronchopulmonary dysplasia (chronic lung disease of infancy): an update for the pediatrician. Clin Pediatr 2002; 41: 77-85.
12. Korhonen P, Tammela O, Koivisto AM, Laippala P, Ikonen S. Frequency and risk factors in bronchopulmonary dysplasia in a cohort of very low birth weight infants. Early Hum Dev 1999; 54: 245-258.
13. Ozkan H, Cetinkaya M, Koksal N. Increased incidence of bronchopulmonary dysplasia in preterm infants exposed to preeclampsia. J Matern Neonatal Med 2012; 25: 1-5.
14. Narasaraju TA, Chen H, Weng T, et al. Expression profile of IGF system during lung injury and recovery in rats exposed to hyperoxia: A possible role of IGF-1 in alveolar epithelial cell proliferation and differentiation. J Cell Biochem 2006; 97: 984-998.

15. Tullus K, Noack GW, Burman LG, Nilsson R, Wretlind B, Brauner A. Elevated cytokine levels in tracheobronchial aspirate fluids from ventilator treated neonates with bronchopulmonary dysplasia. Eur J Pediatr 1996; 155: 112-116.

16. Stahl A, Connor KM, Sapieha P, et al. The mouse retina as an angiogenesis model. Invest Ophthalmol Vis Sci 2010; 51: 2813-2826

17. Hellström A, Engström E, Hård A-L, et al. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. Pediatrics 2003; 112: 1016-1020.

18. Aladağ N. Serum "Insulın-like growth factor-1" düzeyleri ile prematüre yenidoğanın kronik akciğer hastalığı arasındaki ilişki. Uzmanlık Tezi, Trakya Üniversitesi Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Edirne, 2006.

19. Capoluongo E, Ameglio F, Zuppi C. Insulin-like growth factor-I and complications of prematurity: A focus on bronchopulmonary dysplasia. Clin Chem Lab Med 2008; 46: 1061-1066.

20. Murch SH, Costeloe K, Klein NJ, et al. Mucosal tumor necrosis factor-alpha production and extensive disruption of sulfated glycosaminoglycans begin within hours of birth in neonatal respiratory distress syndrome. Pediatr Res 1996; 40: 484-489.

21. Bagchi A, Viscardi RM, Taciak V, Ensor JE, McCrea KA, Hasday JD. Increased activity of interleukin-6 but not tumor necrosis factor-alpha in lung lavage of premature infants is associated with the development of bronchopulmonary dysplasia. Pediatr Res 1994; 36: 244-252.

22. Jónsson B, Tullus K, Brauner A, Lu Y, Noack G. Early increase of TNF alpha and IL-6 in tracheobronchial aspirate fluid indicator of subsequent chronic lung disease in preterm infants. Arch Dis Child Fetal Neonatal Ed 1997; 77: F198-F201.

23. Kotecha S, Chan B, Azam N, Silverman M, Shaw RJ. Increase in interleukin-8 and soluble intercellular adhesion molecule-1 in bronchoalveolar lavage fluid from premature infants who develop chronic lung disease. Arch Dis Child Fetal Neonatal Ed 1995; 72: F90-F96.

24. Shimotake TK, Izhar FM, Rumilla K, et al. Interleukin (IL)-1 beta in TAs from premature infants induces airway epithelial cell IL-8 expression via an NF-kappa B dependent pathway. Pediatr Res 2004; 56: 907-913.

25. Bose CL, Dammann CEL, Laughon MM. Bronchopulmonary dysplasia and inflammatory biomarkers in the premature neonate. Arch Dis Child Fetal Neonatal Ed 2008; 93: F455-F461.

26. Mahieu LM, De Dooy JJ, Ieven MM, Bridts CH, Stevens WJ. Increased levels of tumor necrosis factor-alpha and decreased levels of interleukin-12 p 70 in TAs, within 2 hrs after birth, are associated with mortality among ventilated preterm infants. Pediatr Crit Care Med 2005; 6: 682-689.

27. Jónsson B, Li YH, Noack G, Brauner A, Tullus K. Downregulatory cytokines in tracheobronchial aspirate fluid from infants with chronic lung disease of prematurity. Acta Paediatr 2000; 89: 1375-1380.

28. Paananen R, Husa AK, Vuolteennaho R, Herva R, Kaukola T, Hallman M. Blood cytokines during the perinatal period in very premature infants: relationship of inflammatory response and bronchopulmonary dysplasia. J Pediatr 2009; 154: 39-43.

29. Ambalavanan N, Carlo WA, D'Angio CT, et al. Cytokines associated with bronchopulmonary dysplasia or death in extremely low birth weight infants. Pediatrics 2009; 123: 1132-1141.

30. Jones CA, Cayabyab RG, Kwong KY, et al. Undetectable interleukin (IL)-10 and persistent IL-8 expression early in hyaline membrane disease: A possible developmental basis for the predisposition to chronic lung inflammation in preterm newborns. Pediatr Res 1996; 39: 966-975.

31. Beresford MW, Shaw NJ. Detectable IL-8 and IL-10 in bronchoalveolar lavage fluid from preterm infants ventilated for respiratory distress syndrome. Pediatr Res 2002; 52: 973-978.

32. McColm JR, Stenson BJ, Biermasz N, McIntosh N. Measurement of interleukin 10 in bronchoalveolar lavage from preterm ventilated infants. Arch Dis Child Fetal Neonatal Ed 2000; 82: F156-F159.