ORIGINAL ARTICLE

Cytokine profiles at birth and the risk of developing severe respiratory distress and chronic lung disease

Majeda S Hammoud, Raj Raghupathy[1], Nahla Barakat[2], Hoda Eltomi[1], Deena Elsori[1]
Departments of Pediatrics and [1]Microbiology, Faculty of Medicine, Kuwait University, [2]Department of Neonatology, Maternity Hospital, Kuwait

Background: Neonates with the diagnosis of respiratory distress syndrome (RDS) were studied to investigate possible associations between cytokine levels at birth and developing severe RDS or chronic lung disease (CLD). **Materials and Methods:** This was a cross-sectional study on serum and bronchoalveolar lavage (BAL) samples collected within hours of birth from infants with moderate and severe RDS. Twenty infants with moderate RDS and 20 infants with severe RDS were studied. RDS was diagnosed on the basis of radiographic findings, respiratory distress, and an increasing oxygen requirement. RDS severity was graded based on the radiological findings and Downe's Score. CLD was diagnosed when infants were still on supplemented O_2 by at least 28 days of age. Levels of the cytokines interleukin (IL)-1β, IL-6, IL-8, IL-10, and tumor necrosis factor alpha were measured using enzyme-linked immunosorbent assay. "Statistical analysis was performed using the SPSS for Windows, (SPSS Inc., Chicago, IL, USA)." **Results:** Levels of the proinflammatory cytokines IL-8 and IL-1β were significantly higher in BAL of infants with severe RDS than those with moderate RDS ($P = 0.007$ and $P = 0.02$, respectively). IL-8 levels were also significantly higher in BAL and serum of infants who later progressed to CLD than in those who did not ($P = 0.03$ for both). The IL-8/IL-10 cytokine ratio was significantly higher in the BAL of severe RDS infants than in moderate RDS ($P = 0.01$) and in the serum of infants who progressed to CLD than in those who did not ($P = 0.03$). **Conclusion:** Levels of IL-8 and the IL-8/IL-10 ratio measured soon after birth were associated with severity of RDS as well as progression to CLD. Early measurement of cytokines levels and ratios may contribute to the prognosis and management of RDS and CLD.

Key words: Chronic lung disease, inflammatory cytokines, respiratory distress syndrome

How to cite this article: Hammoud MS, Raghupathy R, Barakat N, Eltomi H, Elsori D. Cytokine profiles at birth and the risk of developing severe respiratory distress and chronic lung disease. J Res Med Sci 2017;22:62.

INTRODUCTION

Respiratory distress syndrome (RDS) due to surfactant deficiency among preterm infants is the most common reason for admission to the neonatal Intensive Care Unit (NICU).[1] Despite advances in antepartum, intrapartum, and neonatal care, RDS remains a major cause of mortality and morbidity among premature infants, especially in developing countries.[2] About 27%–31% of very low birth weight infants die because of RDS or its complications.[2] Up to 40% of those infants who do survive RDS may develop chronic lung disease (CLD) or bronchopulmonary dysplasia (BPD) with further morbidity later in life,[3-6] and it is known that very low birth weight infants (<1500 g) are at high risk for mortality and neonatal morbidities associated with both short-term and long-term complications.

The pathophysiology of RDS is progressive loss of lung volume, intrapulmonary shunt, and deflation instability[7] due to primary surfactant deficiency in addition to an early inflammatory reaction in the lungs.[8] There is growing consensus that intrauterine proinflammatory cytokines probably play an important role in the development of chronic pulmonary complications, but their role in the pathogenesis of RDS is less clear.[9-11] Inflammatory cytokines play important roles in lung damage among preterm infants who

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_1088_15

Address for correspondence: Dr. Majeda S Hammoud, Department of Pediatrics, Faculty of Medicine, P. O. Box: 24923, Safat 13110, Kuwait.
E-mail: m.hammoud@hsc.edu.kw
Received: 03-01-2016; **Revised:** 21-01-2017; **Accepted:** 03-02-2017

subsequently develop CLD.[12-16] In infants who later develop CLD/BPD, increased concentrations of proinflammatory cytokines are detectable in the amniotic fluid,[13,17] and in the bronchoalveolar lavage (BAL) within hours of birth.[8,18]

Although some studies have demonstrated increased levels of proinflammatory cytokines in RDS,[19,20] these have generally compared cytokine levels in RDS versus normal infants. Associations between the severity of RDS and the levels of inflammatory cytokines tested immediately after birth have not been reported. The demonstration of such associations would suggest that early anti-inflammatory treatment may stop the cascade of complications.

The aim of this study was to compare the levels of selected pro- and anti-inflammatory cytokines in the serum and BAL immediately after birth in neonates with moderate versus severe RDS. The objective was to ascertain whether the levels of the proinflammatory cytokines interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) and the anti-inflammatory cytokine IL-10 immediately after birth are significantly associated with developing severe RDS and subsequent CLD.

MATERIALS AND METHODS

Study subjects

This study was approved by the Ethics Committees of Kuwait University and the Ministry of Health (ethical code Reference: VDR/JC/570). The study was explained to parents of the neonates and informed consent was obtained from the parents of the enrolled infants, after full explanation in a clear language about the study's purpose, duration, procedures, and both risks and benefits. We made it clear to the parents that it is their right not to accept the enrollment or to "withdraw" their child from the study at any time. We also emphasized the confidentiality in our studyss

The study samples were obtained at Maternity Hospital, where about 12,000–13,000 babies are delivered annually. Around 3.2% of all the live births are very low birth weight (≤1500 g).

This was a cross-sectional study recruiting all babies admitted to the NICU during the period between January 2007 and July 2007 with the diagnosis of RDS. Inclusion criteria were inborn premature infants equal to or <32 weeks gestation who required intubation and mechanical ventilation for RDS within 6 h of birth. RDS was diagnosed on the basis of radiographic findings, respiratory distress and an increasing FIO_2 requirement. Severity of RDS was based on chest X-ray findings[21] and Downe's clinical Scoring System.[22] Based on that, two groups were classified for the study; those with moderate RDS where there is granularity of the lung with air bronchogram and Downe's score >6 and severe RDS where there is white out a lung with the loss of cardiac borders and Downe's score >8. We did not include infants with mild degree of RDS who did not need intubation and just needed O_2 and supportive therapy (with or without continuous positive airway pressure "CPAP"). Furthermore, neonates with congenital anomalies or possible infections including maternal premature rupture of membranes or possible chorioamnionitis were excluded from the study.

All included newborns received at least one dose of 100 mg/kg of the natural surfactant "Survanta" (Beractant, Surfactant TA, Ross Laboratories, Columbus, Ohio, USA) as a rescue treatment during the 1st day of life. Demographic and clinical characteristics of the infants, that is, gender, maternal diseases, antenatal steroids, antibiotics, fetal distress, and intrauterine growth retardation were recorded. Data on gestational age, birth weight, mode of delivery and Apgar scores at 1 and 5 min were collated [Table 1].

The arterial O_2 saturation aimed for preterm infants in our hospital is between 88% and 94%. Extubation was considered if the infant had satisfactory blood gases, whereas on ventilator with a mean airway pressure of approximately 7 cm H_2O, ventilatory rate ≤25 and FiO_2 ≤0.3.

Table 1: Distribution of demographic and other basic characteristics of included infants

	Moderate RDS (*n*=20)	Severe RDS (*n*=20)	*P*
Gestational age (weeks), mean±SD	29.70±1.52	29.20±1.62	0.44
Weight (mean±SD)	1173.0±257.34	1145.65±203.33	0.71
Cesarean section (%)	16 (80)	17 (85)	1.00
Sex, male (%)	7 (35)	9 (45)	0.75
Antepartum hemorrhage (%)	5 (25)	3 (15)	0.69
Maternal diabetes (%)	4 (20)	3 (15)	1.00
Maternal hypertension (%)	7 (35)	5 (25)	0.73
Antenatal steroids (%)	2 (10)	3 (15)	1.00
Fetal distress (%)	3 (15)	4 (20)	1.00
Apgar score at 1 min (mean±SD)	4.65±1.76	4.25±1.41	0.43
Apgar score at 5 min (mean±SD)	7.25±1.02	7.45±0.89	0.51

RDS = Respiratory distress syndrome; SD = Standard deviation

Sedation, when used, was stopped at least 12 h before extubation and methylxanthines usually started if the infant was <34 weeks gestation. The grouping of infants as moderate RDS and Severe RDS was performed after ventilation was initiated and X-ray was taken. The surfactant was given, and number of surfactant doses was based on the ventilator parameters and the progress of the infants.

Although the definition of BPD or CLD of prematurity, which is oxygen dependency at 36 weeks postmenstrual age, is more widely used,[4] we have used the definition of CLD as supplemental oxygen requirement at 28 days of life in preterm infants who still have respiratory symptoms and chest radiographic changes.[5] This is because we transfer the preterm infants to other Pediatric Department to complete their treatment once they reach ≥2 kg.

Laboratory methods

BAL samples were collected within the 1st h of intubation. 1 ml/kg sterile saline (0.9%) was instilled using a 2 ml syringe through a 5F-gauge feeding catheter placed through the endotracheal tube into the distal right main bronchus. Saline was instilled and then immediately aspirated back into the syringe. This method, recommended by the European Respiratory Society, has been described previously.[12] BAL samples were clarified by centrifugation at 1500 g for 5 min at room temperature.

Approximately 1.5 ml of blood sample was obtained within 6 h of delivery through an indwelling umbilical venous catheter and then centrifuged. Blood and BAL samples were collected before surfactant treatment was given and were stored at 4°C in the NICU and then transported within hours of collection to the laboratory at the Faculty of Medicine, Kuwait University. These samples were then stored at −70°C until they were tested for cytokines by enzyme-linked immunosorbent assay (ELISA).

Bronchoalveolar fluids were tested for the cytokines IL-1 β, IL-6, IL-8, IL-10, and TNF-α using ultrasensitive ELISA (Immunotech, Marseilles, France). Samples were tested in triplicate, and absorbance values read using an ELISA Reader. Accurate sample concentrations of cytokines were determined by comparing their respective absorbencies with those obtained for the reference standards plotted on a standard curve. Detection limits were 5 pg/ml for IL-1 β and IL-8, 1.5 pg/ml for IL-6, 3 pg/ml for IL-10, and 8 pg/ml for TNF-α.

Statistical methods

Statistical analysis was performed using the SPSS for Windows version 18.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean (± standard deviation), or as median and interquartile range as appropriate. Chi-square test was used to assess the statistical differences in categorical variables. The Student's *t*-test was used to assess the differences if variables were normally distributed and the Mann–Whitney U-test when the variables were not normally distributed. Results were considered statistically significant if the value of $P < 0.05$.

RESULTS

A total of 75 infants had been included in the study; 40 of these infants satisfied the inclusion criteria. Twenty infants had moderate RDS and 20 had severe RDS. Thirty-five infants were excluded because of one of the following reasons: incomplete data ($n = 9$), hemolyzed blood samples ($n = 11$), death before 28 days of age ($n = 8$), and evidence of sepsis proved after inclusion ($n = 7$). Table 1 shows the distribution of basic characteristics in the study groups. There was no significant difference between moderate and severe RDS in terms of gestational age, sex, birth weight, type of delivery, antenatal steroids, and Apgar scores. Similarly, the distribution of these characteristics was not significantly different between infants with CLD and infants without CLD.

Table 2 shows the distribution of pro- and anti-inflammatory cytokines among severe and moderate RDS. The serum level of anti-inflammatory cytokines IL-10 was apparently higher among moderate RDS than in severe RDS; although, it did not reach statistical significance ($P = 0.16$). On the other hand, the serum level of the proinflammatory cytokine IL-8 was marginally higher among severe RDS than moderate RDS; this difference was also not significant ($P = 0.15$).

Levels of pro- and anti-inflammatory cytokine levels in BAL samples among the cases of severe and moderate RDS are presented in Table 2. The proinflammatory cytokine IL-8 was present in 7-fold higher levels in severe RDS than in moderate RDS ($P = 0.007$). Similarly, the level of IL-1β in BAL was 9-fold higher in severe RDS than in moderate RDS ($P = 0.02$).

Ratios of proinflammatory cytokines to the anti-inflammatory cytokine IL-10 are presented in Table 3. The IL-8/IL-10 ratio is about five-fold higher ($P = 0.04$) in the serum of infants who later progressed to severe RDS, and this ratio is about eight-fold higher in the BAL of such infants ($P = 0.01$) [Table 3].

Neonates with moderate and severe RDS were followed-up to determine whether they developed CLD. Out of 20 neonates diagnosed with moderate RDS, five (25%) developed CLD, whereas 7 out of 20 cases (35%) diagnosed with severe RDS developed CLD.

Table 2: Levels of cytokines in serum and Bronchoalveolar lavage of neonates with moderate and severe respiratory distress syndrome

	Median (IQR)		*P*
	Moderate RDS	Severe RDS	
Cytokines in serum			
IL-10	9.86 (4.00-16.19)	2.84 (2.25-4.95)	0.16
TNF-α	3.38 (1.97-4.47)	3.35 (2.14-5.21)	0.99
IL-1β	2.70 (1.62-6.47)	4.56 (1.82-13.47)	0.36
IL-6	46.24 (14.87-89.20)	47.10 (8.32-110.76)	0.85
IL-8	387.44 (108.60-923.48)	679.71 (206.53-1596.55)	0.15
Cytokines in BAL			
IL-10	2.92 (1.38-4.54)	3.33 (0.63-5.22)	0.44
TNF-α	0.014 (0.00-1.498)	0.00 (0.00-0.20)	0.51
IL-1β	2.17 (0.28-21.68)	20.16 (0.41-39.05)	0.02
IL-6	243.54 (43.24-543.795)	127.68 (51.40-715.40)	0.99
IL-8	118.20 (25.69-275.67)	870.18 (163.90-1917.9)	0.007

RDS = Respiratory distress syndrome; BAL = Bronchoalveolar lavage; IQR = Interquartile range; IL = Interleukin; TNF-α = Tumor necrosis factor alpha

Table 3: Ratio of pro- to anti-inflammatory cytokines in moderate versus severe respiratory distress syndrome and chronic lung disease versus no chronic lung disease

	TNF/IL-10		IL-1β/IL-10		IL-6/IL-10		IL-8/IL-10	
	Moderate	Severe (NS)	Moderate	Severe (NS)	Moderate	Severe (NS)	Moderate	Severe
Serum	0.3	0.5	0.5	0.9	37	8	51	252*
BAL	0.7	0.9	3.6	4.5	118	131	56	437*
	TNF/IL-10		IL-1β/IL-10		IL-6/IL-10		IL-8/IL-10	
	No CLD	CLD (NS)	No CLD	CLD (NS)	No CLD	CLD (NS)	No CLD	CLD
Serum	0.4	0.4	0.6	1.1	14.1	58.6	87.2	387.3*
BAL	0.8	0.8	3.6	7.5	128.2	114.1	274.9	760.1 (NS)

*P<0.05. NS = Not statistically significant; CLD = Chronic lung disease; BAL = Bronchoalveolar lavage; IL = Interleukin; TNF-α = Tumor necrosis factor alpha

IL-8/IL-10 and IL-6/IL-10 ratios were higher in the serum of infants who later developed CLD (*P* = 0.03 and *P* = 0.05, respectively) and this is statistically significant for the first ratio only, also the IL-8/IL-10 ratio was higher in the BAL of infants who developed CLD (*P* = 0.05) but not statistically significant [Table 3].

Tables 4 and 5 depict the levels of serum cytokines (4) and BAL cytokines (5) at birth in neonates who developed CLD and those who did not. IL-8 levels were significantly higher in the serum (*P* = 0.03) [Table 4] and BAL (*P* = 0.03) [Table 5] of infants who later developed CLD than those who did not. The distribution of other serum cytokines was similar in two groups except for serum IL-10, which showed significantly lower levels in infants who later developed CLD than infants who did not develop CLD (*P* = 0.02) [Table 4]. There was a trend toward higher levels of IL-1β in both the serum and BAL of infants who developed CLD, but the difference was not significant. Levels of other cytokines in BAL were similar in two groups [Table 5].

DISCUSSION

This study was designed to determine whether the levels of pro- and anti-inflammatory cytokines immediately after birth are significantly different between moderate and severe RDS among preterm babies who are intubated, given surfactant, and ventilated. The previous studies have compared the levels of inflammatory cytokines between RDS and normal infants. Our data indicate significantly higher levels of the proinflammatory cytokine IL-8 in BAL immediately after birth in infants with severe RDS than in infants with moderate RDS. This is consistent with studies which reported a strong association between the level of IL-8 and the duration of intubation[20] and supports the notion that this cytokine may contribute to the severity of RDS.

The inflammatory process in the lungs of preterm infants has been linked to prenatal infections such as chorioamnionitis.[23,24] Although we have excluded prenatal infections, the association between the proinflammatory cytokine IL-8 and severity of RDS is of significant interest. In the absence of prenatal inflammation, the immature lungs of preterm infants are probably affected by a combination of postnatal risk factors and events that induce inflammation such as baro- and volutrauma, oxygen toxicity, and hypoxia.[10,11] It is possible that this tissue damage leads to inflammatory reactions, including the production of inflammatory cytokines.[25,26]

It has been suggested that CLD may be due to an inability to regulate inflammation caused by a lack of the

Table 4: Levels of cytokines in serum at birth among neonates with or without chronic lung disease

	Cytokines in serum samples, median (IQR)		*P*
	No CLD	CLD	
IL-10	11.79 (3.40-20.19)	5.48 (1.28-10.13)	0.02
TNF-α	3.21 (2.15-4.65)	3.44 (1.35-4.71	0.74
IL-1β	3.38 (1.89-6.66)	5.90 (1.36-11.09)	0.62
IL-6	46.24 (7.69-95.76)	52.93 (22.47-105.58)	0.47
IL-8	316.00 (76.32-1067.10)	1197.5 (606.36-1523.55)	0.03

CLD = Chronic lung disease; IQR = Interquartile range; IL = Interleukin; TNF-α = Tumor necrosis factor alpha

Table 5: Levels of cytokines in bronchoalveolar lavage at birth among neonates with or without chronic lung disease

	Cytokines in BAL samples, median (IQR)		*P*
	No CLD	CLD	
IL-10	4.33 (1.04-6.10)	2.25 (0.64-3.80)	0.26
TNF-α	0.01 (0.00-0.67)	0.00 (0.00-0.29)	0.40
IL-1β	2.17 (0.30-34.10)	12.67 (1.03-32.57)	0.09
IL-6	216.87 (55.12-744.14)	87.58 (30.38-184.00)	0.12
IL-8	208.85 (62.50-570.18)	747.11 (95.62-1421.05)	0.03

CLD = Chronic lung disease; BAL = Bronchoalveolar lavage; IQR = Interquartile range; IL = Interleukin; TNF-α = Tumor necrosis factor alpha

anti-inflammatory cytokine IL-10.[27] However, some studies have found no association between IL-10 and the period of intubation[8] which has led to doubts on the influence of this cytokine on the development of CLD. In this study, we have demonstrated higher levels of IL-10 in the serum of infants with moderate RDS and the serum of infants who did not develop CLD at 28 days of life. This might suggest a possible role for IL-10 in mitigating inflammatory processes; Increased IL-10 levels may contribute to the observed decrease in IL-8 concentrations. Several previous studies have shown that IL-10 inhibits IL-8 production by monocytes[27,28] and granulocytes.[29] We propose that the anti-inflammatory cytokine IL-10 plays an important role of down regulating the production of proinflammatory cytokines like IL-8. Interestingly, there was no difference in the levels of IL-10 in lavage samples of moderate and severe RDS and between those with CLD and those without CLD. We suggest that this may be because BAL samples were obtained immediately after intubation, perhaps at a stage when inflammatory cytokines had not yet leaked from pulmonary vessels; it is possible that differences similar to that seen in the serum may be observed if lavage samples were collected at the later time point.

As shown by the previous studies,[19,20] we found an association between the level of IL-8 in the serum and lavage of preterm infants who later developed CLD. However, previous studies[16] estimated cytokine levels on days 8–10. Sampling neonates as late as day 8 would have little value in guiding clinical management, as pathophysiological processes might well be underway, possibly even to an irreversible stage. Only one study demonstrated that IL-8 levels in BAL on day 1 was inversely correlated with prematurity and associated with the development of CLD.[15] In this study, the association with IL-8 was detected immediately after birth, and this information can help initiate early management to prevent subsequent respiratory complications. The association between serum IL-8 and developing CLD is consistent with studies that have demonstrated increased concentrations of proinflammatory cytokines in the plasma of ventilated preterm newborns during the 1st day of life.[13] Information such as this, in addition to data on levels of other cytokines and inflammatory mediators, will contribute to better understanding of the pathogenesis of both RDS and CLD.

We suggest that serum and lavage values of IL-8 and IL-8/IL-10 ratios may serve as indicators for the later development of CLD in ventilated preterm infants. This of course needs to be validated in larger sample sizes. Other proinflammatory cytokines including IL-1β, TNF-α, and IL-6 do not appear to be associated with developing severe RDS and CLD.

While the concentrations of cytokines are of interest, it is perhaps more pertinent to compare the ratios of pro-inflammatory to anti-inflammatory cytokines as such ratios are likely to provide information on possible biases toward pro- and anti-inflammatory cytokine dominance. The IL-8/IL-10 cytokine ratio is higher in the serum and BAL of severe RDS than moderate RDS. The IL-8/IL-10 ratio is also higher in the serum and BAL of infants who later developed CLD than in those who did not. If this trend is substantiated in studies on larger sample sizes, such cytokine ratios may be worth exploring as prognostic indicators of progression to severe RDS and CLD.

One of the limitations of our study was using the CLD definition of oxygen dependency at 28 days instead of oxygen dependency at 36 weeks corrected gestational age. This is because we have a crowded unit which usually transfers infants who are oxygen dependent beyond 1 month of age if they are around 2000 g to other pediatric wards and this would require the study to be conducted in two different hospitals, which is logistically difficult.

The sample size in this study is admittedly low; many samples were excluded for various reasons such as collection and/or storage errors; only those subjects were included, for whom both BAL and blood samples were available. Further studies on larger sample sizes ought to focus on the relationship between the level of inflammatory cytokines during the 1st day of birth and developing CLD.

CONCLUSION

If such associations between different cytokines/cytokine ratios and severity of RDS are confirmed, it could translate

into a good prognostic indicator that would guide the management of RDS and CLD, and hence reduce the risk of developing these conditions. Management options can include anti-inflammatory drugs early in the course of the disease which can help prevent CLD and even can prevent the milder changes in the lung parenchyma which may reflect adversely on the health of the child later in life.

Acknowledgments

This study was supported by Kuwait University Research Grant MK01/03. We gratefully acknowledge the assistance provided by the medical and nursing staff in the NICU of Maternity Hospital, Kuwait as well as Dr. Fawaz Azizieh and Mr. Parvez Raut for their excellent technical support.

Financial support and sponsorship

Project Funded by Kuwait University research Grant MK01/03.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Flor-de-Lima F, Rocha G, Guimarães H. Impact of changes in perinatal care on neonatal respiratory outcome and survival of preterm newborns: An overview of 15 years. Crit Care Res Pract 2012;2012:643246.
2. Horbar JD, Carpenter JH, Badger GJ, Kenny MJ, Soll RF, Morrow KA, *et al*. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. Pediatrics 2012;129:1019-26.
3. Bentham JR, Shaw NJ. Some chronic obstructive pulmonary disease will originate in neonatal intensive care units. Paediatr Respir Rev 2005;6:29-32.
4. Jobe AH, Bancalari E. National Institute of Child Health and Human Development/National Heart, Lung, and Blood Institute/Office of Rare Diseases. Workshop summary: Bronchopulmonary dysplasia. Am J Respir Crit Care Med 2001;163:1723-9.
5. Shima Y, Kumasaka S, Migita M. Perinatal risk factors for adverse long-term pulmonary outcome in premature infants: Comparison of different definitions of bronchopulmonary dysplasia/chronic lung disease. Pediatr Int 2013;55:578-81.
6. Latini G, De Felice C, Giannuzzi R, Del Vecchio A. Survival rate and prevalence of bronchopulmonary dysplasia in extremely low birth weight infants. Early Hum Dev 2013;89 Suppl 1:S69-73.
7. Toti P, Buonocore G, Rinaldi G, Catella AM, Bracci R. Pulmonary pathology in surfactant-treated preterm infants with respiratory distress syndrome: An autopsy study. Biol Neonate 1996;70:21-8.
8. 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:F156-9.
9. Kramer BW, Kallapur S, Newnham J, Jobe AH. Prenatal inflammation and lung development. Semin Fetal Neonatal Med 2009;14:2-7.
10. Jobe AH. Antenatal associations with lung maturation and infection. J Perinatol 2005;25 Suppl 2:S31-5.
11. Westover AJ, Moss TJ. Effects of intrauterine infection or inflammation on fetal lung development. Clin Exp Pharmacol Physiol 2012;39:824-30.
12. de Blic J, Midulla F, Barbato A, Clement A, Dab I, Eber E, *et al*. Bronchoalveolar lavage in children. ERS task force on bronchoalveolar lavage in children. European Respiratory Society. Eur Respir J 2000;15:217-31.
13. Ghezzi F, Gomez R, Romero R, Yoon BH, Edwin SS, David C, *et al*. Elevated interleukin-8 concentrations in amniotic fluid of mothers whose neonates subsequently develop bronchopulmonary dysplasia. Eur J Obstet Gynecol Reprod Biol 1998;78:5-10.
14. Kotecha S, Wilson L, Wangoo A, Silverman M, Shaw RJ. Increase in interleukin (IL)-1 beta and IL-6 in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. Pediatr Res 1996;40:250-6.
15. 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:F90-6.
16. Su BH, Chiu HY, Lin TW, Lin HC. Interleukin-8 in bronchoalveolar lavage fluid of premature infants at risk of chronic lung disease. J Formos Med Assoc 2005;104:244-8.
17. Thomassen MJ, Divis LT, Fisher CJ. Regulation of human alveolar macrophage inflammatory cytokine production by interleukin-10. Clin Immunol Immunopathol 1996;80(3 Pt 1):321-4.
18. Murch SH, Costeloe K, Klein NJ, MacDonald TT. Early production of macrophage inflammatory protein-1 alpha occurs in respiratory distress syndrome and is associated with poor outcome. Pediatr Res 1996;40:490-7.
19. 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;52:973-8.
20. Huang HC, Yang MY, Huang CB, Yang KD. Profiles of inflammatory cytokines in bronchoalveolar lavage fluid from premature infants with respiratory distress disease. J Microbiol Immunol Infect 2000;33:19-24.
21. Kero PO, Mäkinen EO. Comparison between clinical and radiological classification of infants with the respiratory distress syndrome (RDS). Eur J Pediatr 1979;130:271-8.
22. Downes JJ, Vidyasagar D, Boggs TR Jr., Morrow GM 3rd. Respiratory distress syndrome of newborn infants. I. New clinical scoring system (RDS score) with acid – Base and blood-gas correlations. Clin Pediatr (Phila) 1970;9:325-31.
23. Lahra MM, Beeby PJ, Jeffery HE. Intrauterine inflammation, neonatal sepsis, and chronic lung disease: A 13-year hospital cohort study. Pediatrics 2009;123:1314-9.
24. Lahra MM, Jeffery HE. A fetal response to chorioamnionitis is associated with early survival after preterm birth. Am J Obstet Gynecol 2004;190:147-51.
25. Speer CP. Neonatal respiratory distress syndrome: An inflammatory disease? Neonatology 2011;99:316-9.
26. Kramer BW. Antenatal inflammation and lung injury: Prenatal origin of neonatal disease. J Perinatol 2008;28 Suppl 1:S21-7.
27. Jones CA, Cayabyab RG, Kwong KY, Stotts C, Wong B, Hamdan H, *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;39:966-75.
28. Méndez-Samperio P, García E, Vázquez A, Palma J. Regulation of interleukin-8 by interleukin-10 and transforming growth factor beta in human monocytes infected with mycobacterium Bovis. Clin Diagn Lab Immunol 2002;9:802-7.
29. Yilma AN, Singh SR, Fairley SJ, Taha MA, Dennis VA. The anti-inflammatory cytokine, interleukin-10, inhibits inflammatory mediators in human epithelial cells and mouse macrophages exposed to live and UV-inactivated Chlamydia trachomatis. Mediators Inflamm 2012;2012:520174.