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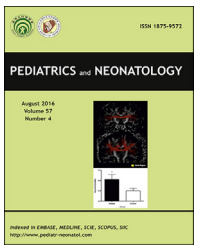

Original Article

Extreme prematurity-associated alterations of pulmonary inflammatory mediators before and after surfactant administration

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Key Words
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Background: The role of prematurity and pulmonary inflammation in the pathogenesis of bronchopulmonary dysplasia (BPD) is very well-defined. However, there is limited knowledge about whether the level of prematurity and surfactant therapy alter the pulmonary cytokines and endothelial growth factor (VEGF).
Methods: This study analyzed the VEGF and cytokines, including interleukin (IL)-1β, IL-6, IL-8, and IL-10, and tumor necrosis factor α (TNF-α) in the tracheal aspirate (TA) of preterm infants obtained before (within 2 h after birth) and 10–12 h after the administration of the first dose of surfactant. TA was collected from 40 infants of 35 or fewer weeks of gestation, including extremely (Group 1, n = 19), very (Group 2, n = 13), and moderate/late (Group 2, n = 8) preterm neonates. In addition to univariate analysis, controlled regression models estimated the association of perinatal factors with the tested parameters and their role in the development of BPD.
Result: We recorded significantly lower post-partum levels of VEGF and higher IL-8, IL-1β, and TNF-α in the TA of Group 1 infants than in Group 2 and 3. Compared to the infants in Group 2 and 3, the post-surfactant increases of pulmonary VEGF, IL-8, IL-10, and TNF-α were more significant in Group 1. All tested parameters in Group 1 and 2 infants, before and after surfactant administration, were comparable. BPD was recorded in nearly 60% of the extremely preterm survivors and was significantly predicted by increased IL-8 before, and elevated TNF-α level after surfactant administration.
Conclusion: This study indicates the association of birth at extremely preterm gestation with reduction in pulmonary VEGF and exacerbation of pro-inflammatory cytokines followed by greater elevation post-surfactant administration levels of VEGF, IL-8, TNF-α, and IL-10 than in neonates born with gestational age of 28–35 weeks.

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1. Introduction

Extreme prematurity is a significant determinant for postnatal oxygen dependence that is predisposed to by mechanical ventilation-associated hyperoxia, leading to lung injury and the development of bronchopulmonary dysplasia (BPD).[1–3] Cytokines and vascular endothelial growth factors (VEGF) are inflammatory mediators that participate in the immuno-biology of BPD, affecting alveolarization, vascular growth, and lung fibrosis.[4–6] Moreover, cytokines activate the alveolar epithelial cells' expression of VEGF[7] that modulates the synthesis of nitric oxide,[8] alveolar cell maturation, and surfactant production.[9,10] To understand the role of inflammatory mediators in the causation of BPD and to evaluate intervention effectiveness for reducing lung damage, several studies have quantified the extent of alteration of cytokines in the tracheal aspirate (TA) of mechanically ventilated extremely preterm neonates.[11–13] Unfortunately, although gestational age (GA) is the principal predictor for postnatal oxygen dependence, there is still limited information about the association between the level of prematurity and the pulmonary inflammatory response. To the best of our knowledge, no study has analyzed the pro-and anti-inflammatory cytokines and VEGF levels in the TA of preterm neonates in a gestational age-dependent manner. Moreover, the role of the level of prematurity in the exogenous surfactant-related alteration of the pulmonary cytokines and VEGF in these infants is not known.

The primary purpose of this investigation was to determine whether the level of prematurity independently from other prenatal factors influenced the alteration of the pulmonary cytokines and VEGF before and after the administration of the first dose of surfactant. Additionally, we analyzed the role of VEGF and cytokines in predicting BPD in models controlled for GA and other pre- and post-natal factors. We hypothesized that extremely preterm infants are born with a pattern of TA VEGF and cytokines that predisposes them to inflammatory lung damage. Therefore, they may respond differently to exogenous surfactant administration as compared to infants born at very and moderately preterm gestations. Our study's findings permit a better understanding of whether prematurity contributes to the activation of pulmonary inflammation before the initiation of mechanical ventilation and if gestational age contributes to the alteration of TA VEGF and cytokine levels in preterm infants after treatment with surfactant.

2. Methods

We designed a prospective cohort study of preterm neonates who were admitted to the Neonatal Intensive Care Unit (NICU) and required intubation to administer exogenous surfactant and mechanical ventilation within the first 2 h of life. Informed consent was required to collect data from the infant's medical record and obtain TA samples during routine nursing care by suctioning the airway of the intubated infants. The Institutional Review Board approved the study at Jersey Shore University Medical Center.

2.1. Collection of TA samples

The TA sampling technique included the insertion of a suction catheter slightly beyond the distal tip of the tube, instillation of 0.5 mL of sterile isotonic saline into the endotracheal tube, and manual ventilation for three breaths followed by suctioning as the catheter was slowly withdrawn. The catheter was cleared of retained secretions by flushing it with an additional 0.5 mL of saline, and the obtained TA was collected in sterile specimen containers. The TA samples were collected twice: (i) within 2 h after birth, before surfactant administration, and (ii) 10—12 h after, during normal airway suctioning. The collected TA was centrifuged for 10 min, and the supernatant was frozen at −80 °C until further assay for VEGF, pro-inflammatory cytokines IL-1β, IL-6, IL-8, and tumor necrosis factor α (TNF-α), and the anti-inflammatory cytokines IL-10 and IL-13.

2.2. VEGF and cytokines testing in TA

The samples were thawed at room temperature for measuring the VEGF and cytokine levels. We assayed the VEGF and cytokines in duplicate using Human VEGF Flex Set (CBA; BD Biosciences, San Diego, CA) and BD FACSArray Bioanalyzer (BD Biosciences, San Diego, CA). VEGF concentrations in the sample were determined from a standard curve ranging from 10 to 2500 pg/mL. The coefficients of variation from inter-and intra-assay precision assessments are <10% for all the assays. We used the Bio-Plex™ Human Cytokine 6-Plex panel (Bio-Rad Laboratories, Hercules, CA, USA) to assay multiple cytokines as per the manufacturer's protocols. Concentrations of IL-1β, IL-6, IL-8, IL-10, IL-13, and TNF-α were measured using commercial test systems (measurable dynamic range 0.2—3200 pg/mL). Due to concern for the plate-to-plate variation in cytokine concentrations, the two samples (before and after administration of surfactant) from each subject were measured in duplicate on the same plate. Cytokine concentrations below the assay detection limits were treated as missing values. The coefficient of variation from inter-and intra-assay precision assessments was <10% for all the assays. VEGF and cytokine findings were combined to ensure the subject and sample-based (first and second) comparability of VEGF and cytokine measurements.

2.3. Demographic and clinical data collection

The data collection tool was standardized to obtain information from the neonatal medical records. The following information was collected: gender (male vs. female), gestational age (GA), birth weight (BW), type of delivery (cesarean or vaginal). Apgar scores (1 and 5 min), type of gestation (multiple or singleton), intrapartum diagnosis of clinical chorioamnionitis and placental pathology (abruption), and antenatal use of steroids and antibiotics. The number of surfactant doses and duration (days) of respiratory support, including mechanical ventilation, continuous positive airway pressure (CPAP), nasal cannula, and preterm morbidities documented in the medical records, were also collected. For further analysis, we categorized patients based on completed weeks of gestation as extremely preterm (<27 weeks, Group 1), very preterm (28 − <32 weeks, Group 2), and moderate or late preterm (32 − <37, Group 3).[14] We used an Apgar score of 3 or less at 1 min to classify preterm infants as being born with asphyxia.[15] BPD was diagnosed if a surviving infant required additional oxygen past 36-weeks of postconceptional age.[16]

2.4. Statistical analysis

We compared the prenatal categorical characteristics of the gestational age-based groups by coding as 1 if an event presented and 0 if not, using Chi-square test statistics. We tested the continuous data for normality using the Shapiro—Wilk *W*-test. ANOVA followed by Tukey test was used to compare parametrically distributed data and the Mann—Whitney U test was used for non-parametrically distributed data, if needed. We also analyzed the significance of the changes in the mean levels of VEGF and cytokines after administration of surfactant in each group using the t-test for dependent samples. All the collected perinatal factors were included in the stepwise regression models to identify their association with VEGF and cytokine levels. We also compared the VEGF and cytokine levels before and after administration of surfactant in the infants who developed BPD versus those who did not. Stepwise logistic regression models were used to identify the role of VEGF and cytokines in the prediction of BPD after controlling for the perinatal and neonatal characteristics, including the number of surfactant doses, and the duration of mechanical ventilation and continuous positive airway pressure (CPAP). Correlation analysis was used to identify the relationships between all tested mediators, before and after surfactant administration, separately in each gestational age group. Normally distributed continuous data were presented as mean and difference in mean (Diff. mean) with 95% Confidence Interval (95%CI). Non-parametric data were presented as median with interquartile range (IQR). We used correlation coefficient (r), regression coefficient (β) and odds ratio (OR) with 95% CI to present the findings from the correlation analysis and stepwise regression models. Two-sided P-values of <0.05 were considered statistically significant. All statistical analyses were conducted using STATISTICA 13.2 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results

Among the 40 studied infants, 19 (47.5%) were in Group 1, 13 (32.5%) in Group 2, and 8 (20.0%) in Group 3. The proportions of male infants in Group 1 (47.4%), Group 2 (38.5%), and Group 3 (50.0%) were comparable (P = 0.97). The GA of preterm infants in Group 3 was not more than 35 weeks. The mean GA and BW of infants in Group 1 was 25.7 weeks (95%CI 25.0—26.3) and 0.848 kg (95%CI 0.746—0.951), in Group 2 it was 29.9 weeks (95%CI 29.2—30.6) and 1.335 kg (95%CI 1.150—1.521), and in Group 3 it was 33.5 weeks (95% CI 32.4—34.6) and 2.125 kg (95%CI 1.826—2.424). The perinatal characteristics of the infants in the study groups were comparable (Table 1). Most of the VEGF and cytokine levels were in the detectable range, except for some IL-13 results before (n = 1) and after (n = 2) the administration of surfactant.

3.1. Group-based comparison of VEGF and cytokines before and after surfactant administration

The distribution of TA levels of VEGF and all tested cytokines collected before and after surfactant administration was normal. Group comparison, before surfactant administration, showed lower VEGF levels and higher IL-8, TNF-α, and IL-1β in Group 1 neonates as compared to Group 2 and 3 but no difference in the TA levels of IL-6, IL-10, and IL-13 (Table 2). Post-surfactant, the within-group comparison of TA levels revealed significantly increased VEGF, IL-6, IL8, IL-10, and TNF-α in each gestational-age group. The post-

Table 1 Group-based comparison of perinatal characteristics.

Characteristics	Study Groups			P-value[a]
	Group 1 (n = 19)	Group 2 (n = 13)	Group 3 (n = 8)	
Multiple gestation, %(n)	21.1% (4)	30.8% (4)	0 (0)	0.22
Placental pathology, % (n)	0% (0)	15.4% (2)	12.5% (1)	0.14
Chorioamnionitis, % (n)	21.1% (4)	0% (0)	0% (0)	0.13
Cesarean section, % (n)	84.2% (16)	76.9% (10)	87.5 (7)	0.79
Steroids, % (n)	47.4% (9)	38.8% (4)	25.0% (2)	0.45
Antibiotics, % (n)	21.1% (4)	7.7% (1)	0% (0)	0.26
Asphyxia, % (n)	15.8% (3)	15.4% (2)	0% (0)	0.46

[a] P value presented group-based comparison of perinatal characteristics using Chi-square test statistics.

Table 2 Group-based comparison of VEGF and cytokine in TA collected before and after surfactant administration.

VEGF and cytokines	Study Groups		
	Group 1 (n = 19)	Group 2 (n = 13)	Group 3 (n = 8)
VEGF			
Before	14.0 (11.4, 16.5)	23.9 (22.0, 25.7)[a]	22.3 (17.6, 27.0)[a]
After	60.6 (49.3, 72.0)	37.3 (34.8, 39.7)[a]	36.8 (32.3, 41.4)[a]
Diff. mean	46.7 (59.9, 33.3)[b]	13.4 (10.4, 16.5)[b]	14.6 (10.2, 18.9)[b]
IL-6			
Before	39.5 (32.8, 46.2)	31.1 (23.2, 41.8)	29.7 (23.0, 36.5)
After	56.7 (42.6, 70.8)	47.2 (41.2, 53.1)	46.9 (42.5, 51.3)
Diff. mean	17.2 (28.1, 6.4)[b]	16.0 (9.6, 22.5)[b]	17.4 (11.0, 23.4)[b]
IL-8			
Before	461.2 (392.6, 529.8)	314.1 (282.5, 345.7)[a]	313.6 (278.9, 348.4)[a]
After	1062.9 (827.4, 1298.6)	483.1 (395.8, 570.4)[a]	497.1 (360.9, 633.3)[a]
Diff. mean	601.8 (781.6, 421.9)[b]	169.0 (99.8, 238.2)[b]	183.5 (44.6, 322.4)[b]
IL-10			
Before	17.5 (15.5, 19.4)	15.5 (14.4, 16.7)	14.8 (13.6, 16.1)
After	28.9 (25.3, 32.6)	21.6 (19.8, 23.5)[a]	21.7 (19.0, 24.2)[a]
Diff. mean	11.5 (7.8, 15.2)[b]	6.8 (4.5, 9.1)[b]	6.1 (4.2, 8.1)[b]
TNF-α			
Before	21.7 (17.1, 26.2)	14.0 (12.6, 15.3)[a]	13.6 (13.2, 14.1)[a]
After	45.1 (34.8, 55.3)	26.1 (22.1, 30.1)[a]	25.7 (21.7, 29.7)[a]
Diff. mean	23.4 (16.6, 30.2)[b]	12.2 (8.7, 15.7)[b]	12.1 (8,1, 16.1)[b]
IL-1β			
Before	19.6 (17.1, 22.0)	13.7 (12.3, 15.2)[a]	14.8 (12.3, 17.3)[a]
After	18.4 (15.9, 21.0)	22.9 (18.9, 26.8)	18.3 (14.5, 22.2)
Diff. mean	−1.1 (−4.8, 2.6)	9.1 (5.5,12.7)[b]	3.6 (0.06, 7.1)[b]
IL-13			
Before	12.2 (11.3, 13.0)	12.2 (11.4, 12.9)	11.9 (11.0, 12.8)
After	12.7 (12.1, 13.3)	13.0 (12.2, 13.8)	12.5 (11.6, 13.4)
Diff. mean	0.53 (0.34, −1.4)	0.87 (0.26, −1.5)	0.63 (0.38, −1.6)

Data represents mean and difference in mean (Diff. mean) with 95%CI of VEGF and cytokine values (pg/mL) for neonates with gestational age of 27 or fewer weeks (Group 1), 28—31 weeks (Group 2), and 32—35 weeks (Group 3).

[a] Displayed significant difference of VEGF and cytokines (P-values <0.002—0.0001) between gestational age groups recorded before and after surfactant administration using ANOVA followed by Tukey test. The tested parameters did not show any statistical differences between Groups 2 and 3.

[b] Displayed a significant difference in mean (P value < 0.05—0.001) of VEGF and cytokines before to those after surfactant administration using t-test for dependent samples.

surfactant increases of VEGF, IL-8, IL-10, and TNF-α in Group 1 was higher than in Groups 2 and 3. The increase of IL-6 after surfactant administration in Groups 1, 2, and 3 was comparable. There was no change in IL-13 in the TA of patients in Groups 1, 2, and 3. The post-surfactant level of IL-1β increased in Groups 2 and 3 but did not change in Group 1.

3.2. Prenatal factors that significantly predicted the post-partum TA levels of cytokines and VEGF

Table 3 presents only the statistically significant findings from the stepwise linear regression models constructed to identify the association of prenatal characteristics with the levels of VEGF and cytokines in TA collected before administration of the first dose of surfactant. Therefore, Table 3 does not include IL-10 as dependent, and gender and placental pathology as independent variables because of the lack of findings suggestive of any significant association. Because of the similarity of the VEGF and cytokine levels, Groups 2 and 3 were combined for inclusion in the regression models and coded (0) for comparison to Group 1 coded (1). As shown in Table 3, extreme preterm birth was associated with decreasing levels of VEGF and increasing levels of IL-6, IL-8, IL-1β, and TNF-α. Prenatal exposure to chorioamnionitis was associated with increased, and steroids with decreased IL-6, IL-1β, and TNF-α, respectively. The IL-13 levels had a direct association with multiple gestations and an indirect association with birth asphyxia.

3.3. Prenatal factors associated with the development of BPD

Among the 19 extremely preterm infants (Group 1), ten were diagnosed with BPD, two died, and at discharge only three required oxygen via nasal cannula. None of the neonates in Groups 2 and 3 were diagnosed with BPD. The extremely preterm infants discharged with BPD had been born at a lower gestational age (median 25.0 weeks, IQR 24—26, P < 0.03), were less likely to have been exposed to

Table 3 Prenatal factors that significantly predicted levels of cytokines and VEGF in TA collected before administration of the first dose of surfactant (**β, 95%CI**).[a]

Coded (1) if "yes"	VEGF	IL-6	IL-8	IL-1b	TNF-α	IL-13
Group 1 vs. Group 0	−0.57 −0.30, −0.84	0.31 0.12, 0.49	0.68 0.43, 0.93	0.34 0.06,0.62	0.33 0.12, 0.55	
Multiple gestations					0.30 0.10, 0.51	0.55 0.22, 0.89
Asphyxia						−0.40 −0.07, −0.74
CA[b]		0.32 0.14, 0.49		0.40 0.12, 0.68	0.48 0.27, 0.69	
Cesarean section		−0.72 −0.54, −0.89				
Steroids		−0.28 −0.11, −0.47	−0.51 −0.26, −0.76		−0.45 −0.24, −0.65	

[a] Regression coefficient (β) presented with 95%CI and significance at P values of <0.01−0.0001.
[b] CA, chorioamnionitis.

intrapartum steroids (30% vs. 85.7%, $P < 0.03$), and were more likely had received 2−3 doses of surfactant than those without BPD (100% vs. 43.0%, $P < 0.03$). We did not find any significant differences in the duration of mechanical ventilation and CPAP, mode of delivery, birth asphyxia, placental pathology and chorioamnionitis, and antenatal use of antibiotics between the infants with and without BPD.

3.4. Comparison of VEGF and cytokine levels based on the development of BPD

Fig. 1 presents a comparison of the levels of VEGF and cytokines in TA collected before (A) and 10−12 h after (B) the administration of the first dose of surfactant in infants who developed BPD versus those who did not. The extremely preterm born infants who later developed BPD had lower VEGF and higher IL-6 levels in their TA that was obtained before the administration of the first dose of surfactant (Fig. 1 A). After the administration of the first dose of surfactant, the levels of VEGF, IL-10, and TNF-α were higher, and IL-1β levels were lower in the TA of the infants who developed BPD (Fig. 1 B). Additionally, the TA IL-8 levels in these infants were high (508.8, 95%CI 442.1−576.6 vs. 321.1, 95% 295.7−339.9, $P < 0.001$) even before the first dose of surfactant and increased further (1388.9, 95%CI 1258.1−1518.6 vs. 477.9, 95%CI 419.9−525.9, $P < 0.00001$) after the administration. We used stepwise logistic regression models to identify the predictive role of TA levels of VEGF and each of the cytokines in the development of BPD, before and after administration of the first dose of surfactant, after controlling for gestational age and the other antenatal and postnatal factors. Regression models revealed reduced BPD risk with antenatal steroids (OR 0.27, 95%CI 0.08, 0.94), and increased risk with IL-8 levels before (OR 1.04, 95%CI 1.01, 1.07) and TNF-α levels after surfactant administration (OR 1.12, 95%CI 1.01%, 1.24%). None of the other factors in the regression model, such as gestational age, chorioamnionitis, number of surfactant doses, and duration of mechanical ventilation, were independently associated with the development of BPD in extremely preterm born infants.

4. Discussion

This study has demonstrated the pattern of the gestational age-associated, early post-natal levels of VEGF and cytokines in the TA of preterm born infants, before and 10−12 h after the administration of the first dose of surfactant. We recorded significantly lower post-partum TA levels of VEGF in extremely preterm born neonates than in their more mature counterparts, which could reflect the extent of endothelial cell damage, critical to the inflammation and post-respiratory distress recovery.[9,10] Moreover, birth at extremely preterm gestation was associated with the increase of IL-8, IL1β, and TNF-α, which could characterize the pulmonary inflammatory response that exacerbates the influx of neutrophils, possibly due to more significant pulmonary cellular and oxidative damage.[17] The post-surfactant increases of pulmonary VEGF, IL-6, IL-8, IL-10, and TNF-α were more significant for VEGF, IL-8, TNF-α and IL-10 in the extremely preterm infants than in those born with a gestational age of 28−35 weeks. To our knowledge, no study has analyzed the post-surfactant alteration of TA levels of VEGF and cytokines in neonates in association with the level of prematurity. Experimental and animal research data revealed immune−modulatory properties of surfactant showing a post-surfactant increase of TA concentration of VEGF,[18] lack of alteration[19] or inhibition[20] of pro-inflammatory cytokines in lipopolysaccharide-induced acute respiratory distress syndrome models. Knowledge regarding the exact mechanisms by which exogenous surfactants modulate cytokine production is still limited. Possibly surfactant-associated activation of mononuclear cells[21] augments the production of cytokines, which conceivably supports our findings of increased levels of pro-inflammatory cytokines after surfactant administration. As in our study, post-surfactant treatment increase of the anti-inflammatory cytokine (IL-10) in bronchoalveolar fluid of pediatric patients has been reported.[22] We did not find any meaningful explanation for the gestational age-based discrepancy of the post-surfactant alteration of IL-1β. The correlation analysis performed to explain this finding revealed an association of the post-surfactant levels of IL-1 β

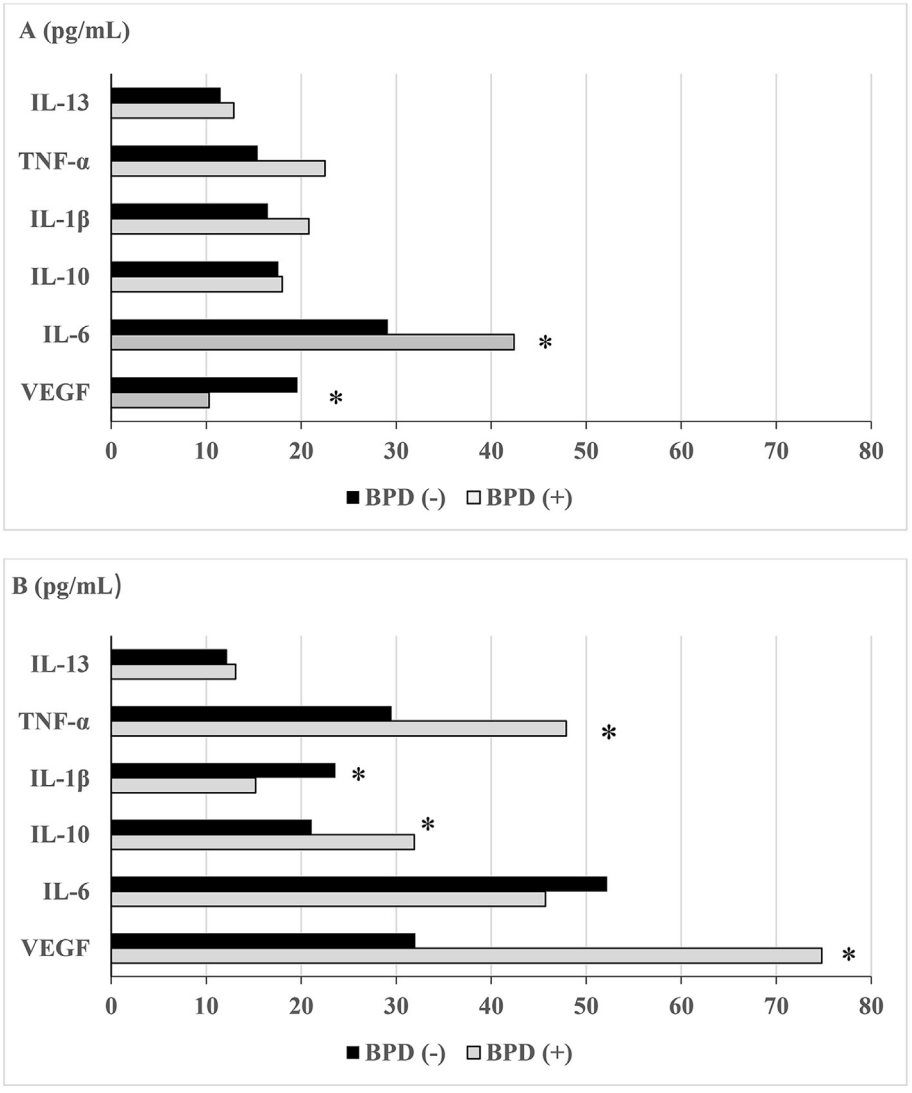

Fig. 1 BPD-associated levels of VEGF and cytokines before (A) and after surfactant (B) administration. * displayed a significant difference (P-value <0.01—0.001) of mean levels of VEGF and cytokines before (Fig. 1 A) and after surfactant (Fig. 1 B) administration in patients with BPD (+) and without BPD (−).

and all other mediators in the TA of the extremely preterm neonates but not in those born with gestational age of 28—35 weeks. The mediators, including VEGF ($r = -0.76$, $P < 0.01$), IL-8 ($r = -0.62$, $P < 0.02$), and IL-10 ($r = -0.64$, $P < 0.02$) that showed a more prominent increase after the administration of surfactant, were negatively correlated with IL-1β. Such findings could, to some extent, clarify the recorded lack of alteration of IL-1β after surfactant administration in the extremely preterm neonates. Nevertheless, the increase of IL-1β in the more mature neonates confirmed the pattern of the pulmonary inflammatory response associated with the administration of the first dose of surfactant.

In addition to the gestational age-stratified analysis, we also used controlled regression models to identify the role of antenatal factors in altering the TA levels of VEGF and the tested cytokines. Exposure to clinical chorioamnionitis was found to be associated with increased TA levels of IL-6, IL-1β, and TNF-α, which is comparable to the previous studies that reported elevation of IL-1α, IL-1β, and IL-8 within 48 h[23] and IL-8 on day one[24] in very preterm born infants. We also found lower IL-6, IL-8, and TNF-α in the TA of infants exposed to antenatal steroids, lower IL-13 in the TA of neonates born with asphyxia, and lower IL-6 in the TA of those delivered by cesarean section. The association of the anti-inflammatory effect of partial and complete antenatal glucocorticoid therapy in reducing inflammatory mediators on Day 1 in the blood of preterm infants born before 28 weeks of gestation has been previously reported.[25] The anti-inflammatory properties of glucocorticoids may predispose to the decrease in prematurity-related respiratory pathology.[26,27] There is limited information regarding the role of asphyxia and mode of delivery in the alteration of pulmonary inflammatory mediators. We found only one report that showed cesarean delivery-associated reduction of toll-like receptors (1—2) that trigger TNF-α and IL-6 responses at birth.[28]

In this study, we assessed the alterations in the levels of cytokines and VEGF in the TA for predicting BPD in controlled

regression models. Analogous to the national data,[29] nearly 60% of the extremely preterm born infants in our study developed BPD, and none were born at gestational age of 28—35 weeks. Previous studies have identified the predictive role of various pulmonary inflammatory mediators for the development of BPD in preterm born infants, including postpartum increased pulmonary IL-8,[30,31,32] TNF alpha,[33] IL-1β,[33] and IL-6.[31,33] However, none of the studies analyzed the post-surfactant administration levels of cytokines for predicting chronic lung pathology in preterm born infants. Our study showed that not only the elevation of IL-8 but also the post-surfactant increase of TNF-α may be relevant for the prediction of BPD. We did not find any independent association of the alteration of VEGF with the development of BPD. However, the existing data in this regard are inconsistent. Studies have reported progression to BPD in association with the elevation[34] or reduction[35] of VEGF within the first few days of postnatal life. Similar to other investigators,[36] we found an almost 70% reduction in the likelihood of developing BPD if antenatal steroids had been used. The results from the regression model in our study did not show the association of chorioamnionitis in predicting BPD, independently from the gestational age. Although the use of regression modeling strengthens the investigation of BPD-associated factors, the limited sample size reduces the ability to make a meaningful conclusion. A meta-analysis of nearly 60 heterogeneous studies published in 2012 reported on the association of chorioamnionitis with BPD,[37] but a meta-regression analysis published in 2019 revealed that gestational age had a modulatory role in the prediction of BPD in chorioamnionitis-exposed infants.[38] We believe that the use of regression analysis significantly strengthens the precision of the study findings by presenting an independent association of the immaturity in the alteration of the VEGF and cytokines in the TA before and after surfactant administration and the prediction of BPD development. At the same time, we want to acknowledge the limitations of our study. The utilization of a convenient non-probability sample, although considered suitable for the enrolment of subjects with similar characteristics,[39] could be associated with a selection bias. To reduce the effect of selection bias on the study findings, we collected TA samples prospectively from each infant to conduct paired comparisons without loss of follow-up. An unequal number of participants in the gestational age sub-groups is a study limitation associated with decreased statistical power. We combined Groups 2 and 3 as they had comparable TA levels of the tested mediators for inclusion in the regression model to improve the precision of the obtained findings. We recognize that the small number of BPD cases reduces the possibility of making of significant conclusions. Therefore, the BPD-associated findings were presented only in the result and discussion sections of the manuscript. However, the obtained results on the alteration of cytokines post-surfactant administration could contribute to the existing knowledge on the role of surfactants in the pathophysiology of BPD.

5. Conclusion

Birth at an extremely preterm gestation is characterized by reduced pulmonary production of VEGF and increased release of pro-inflammatory mediators. Post-administration of surfactant, it is associated with a more prominent increase of VEGF, pro-inflammatory (IL-8, TNF- α) and anti-inflammatory (IL-10) cytokines than in more mature preterm neonates.

Contributor's statement

All three co-authors have made substantive intellectual contribution to the study (conception and design, acquisition of data, or analysis and interpretation of data, drafting of the manuscript or revising it critically for important intellectual content) and take public responsibility for the appropriate portions of the content of the manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

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