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Serum Levels of Interleukin-10 as a Potential Indicator of Outcome in Premature Neonates with Respiratory Distress Syndrome

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ABSTRACT

Background: Respiratory distress syndrome (RDS) is one of the major causes of mortality in preterm newborns. Regarding the close association between inflammatory pathways and the occurrence of RDS, the present study aimed to determine the serum level of interleukin-10 (IL-10) in premature neonates with and without RDS.

Methods: In this case-control study, the serum level of IL-10 was assessed by immunoassay method in both groups of 30 premature neonates with RDS and 28 newborns without RDS as controls. Statistical analysis was conducted to compare potential variations among premature neonates with or without RDS.

Results: The mean level of IL-10 was significantly higher in neonates with RDS, compared to the non-RDS group (41.20±75.78 pg/ml versus 7.73±14.31 pg/ml, P=0.014, respectively). The IL-10 was significantly higher in non-survived neonates, compared to survived newborns (178.76±69.67 pg/ml vs. 6.81±4.06 pg/ml, P=0.0001). In multivariate linear regression analysis, the presence of RDS was associated with the increased level of serum IL-10 (Beta=1.038, P=0.001).

Conclusion: This study revealed that the increased serum levels of IL-10 in premature neonates with RDS might be considered an early indicator of fatal outcomes with high specificity.

Keywords: Interleukin, Neonate, Prematurity, Respiratory distress syndrome

Introduction

Neonatal respiratory distress syndrome (RDS) is a syndrome due to pulmonary immaturity and pulmonary surfactant deficiency affecting about 1% of newborns worldwide (1, 2). The lipid-dense surfactant consists of four specific proteins involved in the exchange of gas. By reducing the production of surfactants, atelectasis throughout the pulmonary parenchyma causes a cytokine-mediated inflammatory response (3-5).

To understand the pathophysiology and management of RDS, extensive studies have been conducted leading to changes in infant mortality with RDS (6). In this regard, several complementary therapies have been introduced to improve the survival of extremely premature RDS infants, including the use of antenatal steroids to enhance pulmonary maturity, early use of surfactants and fluid, as well as electrolyte management (7). Despite all supporting protocols, serious morbidities, such as septicemia, apnea, and intraventricular hemorrhage, are still likely to occur (8). The strategic goals for managing these infants include focusing direct attention on anticipating and minimizing these complications, thereby preventing premature delivery.

The pathophysiological basis of RDS is the inflammatory response, and cytokines may play a major role in its progression. Among them, interleukin-10 (IL-10) which is produced by almost all subsets of leukocytes exerts its immunosuppressive impact by the influence of T-helper cells to reduce tissue damage caused by

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unrestrained inflammatory responses (9-11). Some studies have shown an increase in the serum IL-10 levels in preterm infants (12). An inverse association between the IL-10 levels in the cord blood has also been reported (13). However, the role of this cytokine in the progression of RDS has remained uncertain. Because of the close association between inflammatory pathways and the occurrence of RDS, the crucial role of IL-10 in premature neonates is now expected. The present study was therefore intended to determine the difference in the serum levels of IL-10 in premature neonates with or without RDS.

Methods

Study participants

This case-control and single-center study (the neonatal intensive care unit [NICU] of 17th Shahrivar Children's Hospital in Rasht, Iran) was conducted from 2015 to 2018. In this study, 58 premature neonates delivered during the study from the postnatal ward of the hospital were randomly allocated into two groups of 30 neonates with RDS as a case group and 28 newborns without RDS as a control group. The flow chart of our study is depicted in Figure 1. Study details were clarified to the parents, and informed written consent was obtained from the parents of hospitalized infants to participate in the study. The trial protocol was approved by the Ethics Committee of Guilan University of Medical Sciences, Rasht, Iran (Grant number: 93041003 and code of ethics: IR.GUMS. REC.1396.3344).

Inclusion and Exclusion Criteria

The Inclusion criteria were preterm neonates born at <37 weeks of gestational age, evidence of RDS at birth, and chronological age less than 24 h. Briefly, RDS was diagnosed on the basis of a typical radiological pattern with reduced air content, lung reticulogranular pattern, and air bronchograms; no signs of infection; and increased oxygen dependence during the first 24 h. Moreover, the severity of RDS was evaluated by the Downes score including grunting, retractions, air exchange, respiratory rate, and cyanosis (Table 1) (14).

On the other hand, those neonates with a history of TORCH infections, *intrauterine growth retardation*, maternal acute infections, prolonged rupture of membranes, congenital anomalies, twin to twin transfusion syndrome, and asphyxia were excluded from the study, followed by the neonates of the parents unsatisfied with participating in the study. Accordingly, non-RDS neonates of <37 weeks of gestational age hospitalized in the NICU were selected as the control group. The basic characteristics, including gender, age, gestational age, surfactant therapy, length of hospital stay, and neonatal death, were collected through a review of the recorded files.

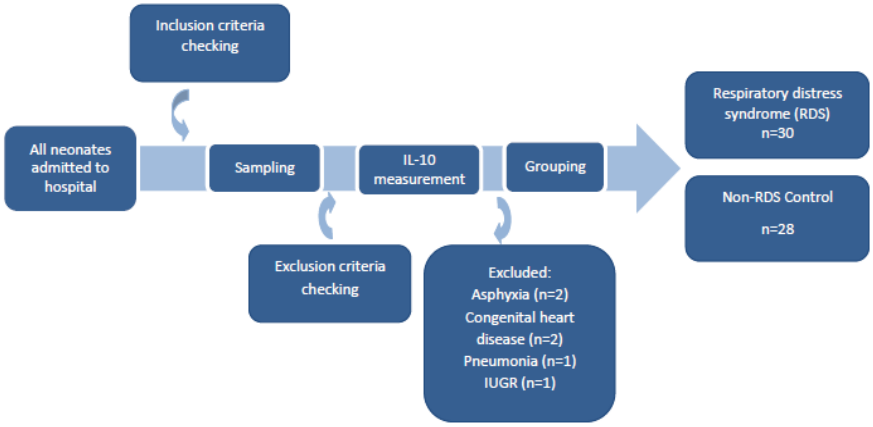

Figure 1. Flow chart of grouping method of our research

Table 1. Downes clinical score system for the evaluation of the severity of RDS

Test/ Score	0	1	2
Grunting	None	Audible by stethoscope	Audible by ear
Retractions	None	Mild	Severe
Air exchange	Good bilateral air entry	Decreased	None
Respiratory rate	<60 per min	60-80 per min	>80 per min
Cyanosis	None	In room air	In 40% FIO_2

Total score: <4 No Respiratory distress, 4-7 Respiratory distress, >7 Respiratory failure

Measurements of IL 10

Blood samples for IL-10 measurement were drawn from an antecubital vein within the first 24 h of birth and centrifuged at 1000×g for 10 min immediately. The serum aliquots were then stored at -70°C. The IL-10 levels were determined using the human IL-10 Quantikine® immunoassay Kit (eBioscience, Vienna, Austria) as instructed by the manufacturer. Briefly, the IL-10 in the samples was bound to the immobilized antibody in each well. An enzyme-linked monoclonal antibody specific to IL-10 was added to the wells. Following washing, a substrate was added and the color was changed in proportion to the amount of IL-10. The reaction was stopped with sulfuric acid, and the absorbance was measured at 450 nm. The reported limit of the detection of the assay was 1.0 pg/ml; in addition, intra- and inter-assay coefficients of variations were reported to be 3.2% and 5.6%, respectively.

Statistical Analysis

The obtained data were analyzed in SPSS software (version 26.0) (SPSS Inc., Chicago, IL), and the results were presented as mean±SD for quantitative variables and summarized by absolute frequencies and percentages for categorical variables. Categorical variables were compared using the chi-square test or Fisher's exact test. Regarding the between groups, the quantitative variables were also compared employing the t-test or Mann-Whitney U test. The correlations between the continuous data were estimated utilizing Spearman's ϱ correlation coefficient. P-values of 0.05 or less were considered statistically significant.

Results

A total of 58 premature neonates were included in the study on the basis of the inclusion criteria. As summarized in Table 2, there was no difference between the two groups with or without RDS in the distribution of basic characteristics, including age, gender, and birth weight. However, in premature neonates with RDS, the mean gestational age was significantly lower than that in those without RDS (32.03±2.14 weeks vs. 33.82±2.34 weeks, P=0.004). Therefore, the correlation between IL-10 and the stratified gestational age was investigated. Spearman's ϱ correlation test showed no significant correlation between the gestational age and the cytokine concentration (Table 3).

Mean serum IL-10 levels were significantly higher in the RDS patients, compared to those without RDS (41.20±75.78 pg/ml vs. 7.73±14.31 pg/ml, P=0.024). Moreover, the mean values of IL-10 levels in the RDS group for f/m were 43.99+75.49 pg/ml vs. 39.07+78.25 pg/ml (P=0.863) and in non-RDS for f/m were 13.73+24.79 pg/ml vs. 4.89+2.75 pg/ml (P=0.317), respectively (Table 4). Although females had higher IL-10 levels in both groups, compared to males, there were no significant differences among females in terms of the IL-10 levels.

Overall, 83.3% of the RDS neonates received surfactant. Both groups with and without RDS were also statistically similar to those receiving corticosteroids (43.3% vs. 57.1%, P=0.297) with mean hospital stay durations of 12.47±8.14 vs. 10.25±4.50 days (P=0.228). Cytokine level in the RDS neonates born from corticosteroid-receiver mothers was 46.30+84.95 pg/ml, compared to the non-corticosteroid receivers (37.30+70.42 pg/ml, P=0.753), and in non-RDS, the corresponding values were 9.33+15.73 pg/ml and 5.59+3.96 pg/ml (P=0.504). In neonates born to mothers receiving steroids, the IL-10 concentrations were higher than the non-steroid receiver (Table 3); however, no statistical significance in the IL-10 levels was found between both groups. Despite the high levels of cytokines in maternal infants treated with corticosteroids, there was no correlation between the RDS (rho=0.027, P=0.887) and non-RDS groups (rho=0.054, P=0.786) regarding the IL-10 levels.

According to Downes score, RDS patients were classified into mild and severe groups. Although the mean serum IL-10 levels were higher in severe RDS patients, compared to those with mild RDS (47.51±81.76 pg/ml vs. 9.65±6.04 pg/ml,

Table 2. Baseline characteristics and outcome in neonates with and without RDS

Item	Neonates with RDS	Neonates without RDS	P-value
Gender Female (%)	13 (43.3)	9 (32.1)	0.384
Age (Hours)	11.83±5.47	12.79±4.83	0.497
Mean birth weight (Grams)	1993.33±543.17	2242.14±540.16	0.096
Mean gestational age (Weeks)	32.03±2.14	33.82±2.34	0.004
Use of surfactant (%)	25 (83.3)	0 (0)	0.001
Mother treated with steroids (%)	13 (43.3)	16 (57.1)	0.297
Hospitalization (Days)	12.47±8.14	10.25±4.50	0.228
Mortality (%)	6 (20.0)	0 (0)	0.013

Abbreviations used: RDS, Respiratory distress syndrome.

Table 3. Correlation of interleukin-10 in RDS and non-RDS groups after stratifying by gestational age

	No of patients		IL10	G A	Spearman's $_{\rho}$, P-value	
	RDS	non-RDS	P-value	P-value	RDS	non-RDS
All cases	30	28	0.014	0.004	-0.285, 0.127	-0.118, 0.548
>28 weeks	29	26	0.034	0.000	-0.217, 0.258	-0.185, 0.366
>30 weeks	22	26	0.092	0.010	-0.119, 0.599	-0.185, 0.366
>32 weeks	13	20	0.068	0.006	-0.146, 0.635	0.027, 0.908
>33 weeks	8	19	0.176	0.091	0.082, 0.846	0.184, 0.452
>34 weeks	3	16	0.467	0.478	0.866, 0.333	0.178, 0.510
>35 weeks	2	7	0.558	1.000	-	-

The figures are the "P-value" obtained statistically by the Mann-Whitney U test

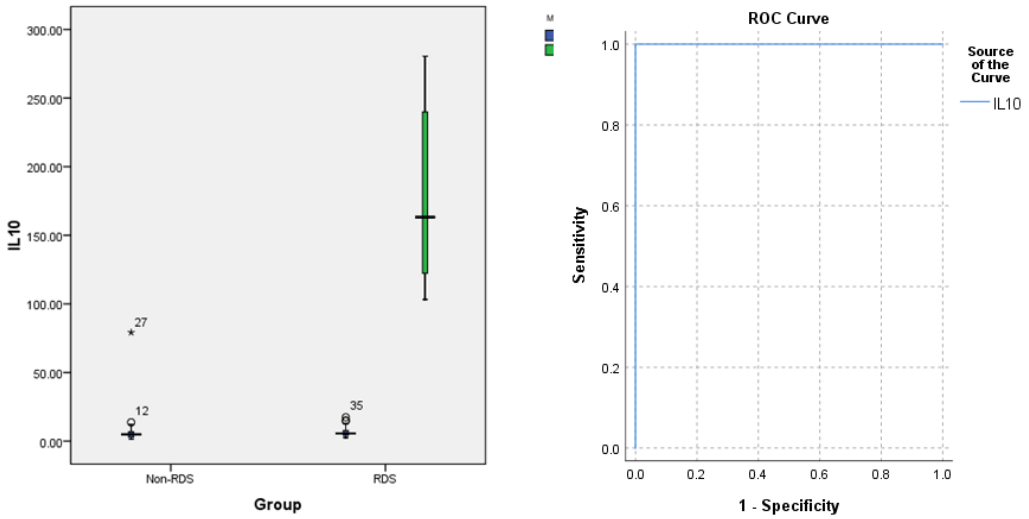

Figure 2. A: Serum levels of IL-10 in RDS and control group based on mortality,
B: Receiver operating characteristic curve analysis of IL-10 in RDS neonates (Area under the curve=1.00, P=0.0001).

P=0.746), it showed no correlation with RDS severity (rho= -0.067, P=0.724).

Regarding mortality, 20.0% of patients with RDS and none of those in the non-RDS group died (P=0.013). As depicted in Figure 2, the level of IL-10 was significantly higher in non-survived neonates, compared to survived newborns (178.76±69.67 pg/ml vs. 6.81±4.06 pg/ml, P=0.0001). In premature neonates with RDS, a strong positive correlation was revealed between the mortality rate and the serum levels of IL-10 (rho=0.693, P<0.001). Receiver operating characteristic (ROC) curve analysis under the nonparametric assumption showed that the IL-10 was a powerful indicator for the diagnosis of fatal outcomes with high specificity in RDS neonates (Area under the curve=1.00, P=0.0001). Finally, in the linear regression analysis of all baseline variables, only the occurrence of death in RDS was associated with increased serum IL-10 (Standardized Beta=1.038, Model R2= 0.874; P=0.001).

Table 4. Level of interleukin-10 according to the baseline characteristics

College	RDS (pg/ml)	Non-RDS(pg/ml)	P value
Gender			
Female	43.99±75.49	13.73±24.79	0.198
Male	39.07± 78.25	4.89±2.75	0.091
Corticosteroid			
Yes	46.30±84.95	9.33±15.73	0.148
No	37.30± 70.42	5.59±3.96	0.082
Gestational age			
<32 weeks	66.48±93.93	6.26±3.62	0.018
>32 weeks	8.14± 4.97	8.31±16.88	0.966
Mortality			
Yes	178.76±69.67	-	-
No	6.81± 4.06	7.73±14.31	0.763

Abbreviations used: RDS, Respiratory distress syndrome.

Discussion

This study was designed to determine whether the levels of regulatory cytokine in preterm infants with RDS are associated with their clinical outcomes. Some authors assessed the levels of inflammatory cytokines in RDS infants and emphasized their role in the severity of RDS. Recently, investigations have focused on gene polymorphisms as responsible for cytokine alteration associated with RDS progression or prevention. In a study by Jin X et al. on the Chinese population, more expression of IL-10, following up-regulation of its gene, was associated with a reduced rate of acute RDS development and mortality (15). In another study by Capasso et al. on Italian preterm infants, the risk of RDS was significantly lower in preterm infants with higher expression of IL-10 -1082 G/A polymorphism (16). Conversely, Gong et al. found that the mutations in the IL-10 gene in patients with RDS were associated with reduced disease severity upon admission and lower 60-day mortality rates (17).

Along with the presence of gene polymorphisms, the bronchoalveolar and serum levels of IL-10 have also changed in relation to RDS in infants. Complexity was defined in the detection of regulatory cytokines in bronchoalveolar lavage (BAL) fluid in the preterm population due to differences in sample collection and processing techniques. In addition, IL-10 levels in BAL samples were not detectable in about 10% of patients with RDs which led us to choose blood samples (18). In line with our observation, Li et al. in their English abstract demonstrated a significant increase in the plasma levels of this marker in patients with RDS, compared to normal controls (19). The important point of the present study was that the increase in IL-10 remained in RDS patients even after adjusting the baseline characteristics. Indeed, the change in IL-10 is caused by the presence of certain gene polymorphisms and other unknown mutations that should be investigated in the future.

The association with disease severity derived from Downes score is in line with the findings of a previous report; however, IL-10 levels were higher in cases with severe RDS, compared to mild forms. In a study conducted by Blanco-Quirós A et al., cord blood IL-10 levels were related to gestational age which was down-regulated in full-term newborns (20). Despite reports indicating a correlation between IL-10 concentrations and gestational age (20), the Spearman's $_{\varrho}$ correlation test showed no significant correlation between the gestational age and the cytokine concentration. These findings are in agreement with the results of other researchers who showed no significant correlations of IL-10 and IL-10 family levels with gestational age (21, 22). It might be assumed that these high serum IL-10 levels in RDS infants indicate the biological immaturity of the fetus or cytokine immunomodulatory effect.

The association between IL-10 and all baseline variables was analyzed in linear regression models. Among all, only the mortality in RDS patients was associated with increased serum IL-10. As mentioned before, IL-10 based on its cellular source and the microenvironment is a regulatory cytokine that contributes to the maintenance of immune homeostasis. It has been shown that preterm infants with RDS had higher IL-10 levels of cord blood than preterm infants without RDS (20). Additionally, higher baseline levels of IL-10 were associated with higher morbidity and mortality in both neonates and adult patients (23, 24). This evidence emphasizes the role of various mediators in the blood of ventilated preterm newborns to develop RDS. This increased IL-10 in premature infants with RDS might be considered an ameliorating mechanism to a balanced inflammatory condition in the lung parenchyma of ventilated neonates.

The performed ROC curve as a visual index of the accuracy of the assay showed that the IL-10 levels could be indicative of poor prognosis in RDS patients. This is a sensitive, specific, as well as not very expensive method to detect RDS severity, particularly in resource-restricted settings.

Conclusion

In conclusion, an increased level of IL-10 is expected in premature neonates with RDS. The IL-10 should be considered an early indicator for the diagnosis of neonatal RDS with usefulness for predicting infantile risk of mortality, compared to other known factors, such as age or weight.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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