Clin. Lab. 2022;68:XXX-XXX

ORIGINAL ARTICLE

Correlations of Serum 1,25-Hydroxy Vitamin D3 level with Severity and Prognosis of Neonatal Respiratory Distress Syndrome

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SUMMARY

Background: **This study was done to explore the correlations of serum 1,25-hydroxy vitamin D3 [1,25-$(OH)_2$-$VitD_3$] level with severity and prognosis of neonatal respiratory distress syndrome (NRDS).**
Methods: **The clinical data of 145 NRDS children admitted between April 2019 and June 2020 were retrospectively analyzed. The subjects were comprised of 76 boys and 69 girls. Based on NRDS severity, they were assigned into mild group (n = 82) and severe group (n = 63), and their general data were compared. The independent factors affecting NRDS severity were evaluated by multivariate logistic regression analysis. The correlations of serum 1,25-$(OH)_2$-$VitD_3$ level in NRDS children with NRDS severity and other related blood test indices were analyzed using Spearman's and Pearson's methods. On the basis of multivariate analysis results, a prediction model was established using R3.6.0 software, which was validated by the receiver operating characteristic (ROC) curve and consistency index. The association between serum 1,25-$(OH)_2$-$VitD_3$ level and prognosis was evaluated through Kaplan-Meier curve.**
Results: **The severe group had higher groups of children with a gestational age < 39 weeks, spontaneous delivery, selective cesarean section, intrauterine distress, intrauterine infection, maternal hypertension, maternal dyslipidemia, and older maternal age than the mild group ($p < 0.05$). Procalcitonin, C-reactive protein, activin-A, Clara cell protein-16, interleukin-18, and IL-10 were significantly higher in the severe group than those in the mild group ($p < 0.05$), while serum bicarbonate, 1,25-$(OH)_2$-$VitD_3$, vitamin A, and IL-8 were significantly lower in the severe group than those in the mild group ($p < 0.05$). Multivariate logistic regression analysis results exhibited that gestational age < 39 weeks, lower 1,25-$(OH)_2$-$VitD_3$ and vitamin A levels, higher activin-A and CC-16 were independent risk factors for severe NRDS ($p < 0.05$). ROC curve analysis revealed that the area under the ROC curve of the model was 0.785, and the prediction accuracy was 88.08%. Serum 1,25-$(OH)_2$-$VitD_3$ level was significantly negatively correlated with NRDS severity ($r = -0.287$, $p < 0.001$), activin-A ($r = -0.073$, $p < 0.001$), and CC-16 ($r = -0.098$, $p < 0.001$), but positively correlated with vitamin A ($r = 0.009$, $p < 0.001$). Kaplan-Meier curve revealed that the survival rate of NRDS children with a high 1,25-$(OH)_2$-$VitD_3$ level was significantly higher than that of children with a low 1,25-$(OH)_2$-$VitD_3$ level ($p < 0.001$).**
Conclusions: **1,25-$(OH)_2$-$VitD_3$ is an independent influencing factor for the severity of NRDS and may affect the prognosis of NRDS children.**
(Clin. Lab. 2022;68:xx-xx. DOI: 10.7754/Clin.Lab.2022.220153)

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Manuscript accepted April 17, 2022

KEYWORDS

neonatal respiratory distress syndrome, 1,25-hydroxy vitamin D3, severity, prognosis

INTRODUCTION

Neonatal respiratory distress syndrome (NRDS) is a clinical syndrome of progressively aggravating respiratory distress shortly after birth due to pulmonary surfactant deficiency [1]. It is primarily manifested as progressive dyspnea, refractory hypoxemia, diffuse infiltrative shadows in both lungs in clinic, and characterized by rapid onset, fast progression, difficulty in treatment, and a high mortality rate, severely threatening the life and health of children [2-4]. 1,25-hydroxy vitamin D3 [1,25-$(OH)_2$-$VitD_3$] is a vital active form of vitamin D3. According to a previous study [5], 1,25-$(OH)_2$-$VitD_3$ is closely associated with the initiation of respiratory diseases, and patients lacking 1,25-$(OH)_2$-$VitD_3$ have a higher chance of developing bronchial asthma and respiratory tract infection than those with a normal 1,25-$(OH)_2$-$VitD_3$ level. A recent study has revealed that 1,25-$(OH)_2$-$VitD_3$ plays an important role in facilitating pulmonary maturation and pulmonary surfactant synthesis [6], suggesting that serum 1,25-$(OH)_2$-$VitD_3$ level is associated with the initiation of NRDS. The lack of 1,25-$(OH)_2$-$VitD_3$ more easily leads to bronchial asthma and recurrent respiratory tract infections [7]. Besides, 1,25-$(OH)_2$-$VitD_3$ has been reported to play a key role in promoting lung maturation and pulmonary surfactant synthesis [8]. The aerosol inhalation of 1,25-$(OH)_2$-$VitD_3$ can facilitate the synthesis and secretion of pulmonary surfactant in neonatal rats [9], and the lack of pulmonary surfactant is one of the important causes for NRDS [10]. Taken together, serum 1,25-$(OH)_2$-$VitD_3$ deficiency in neonates may be related to the occurrence of NRDS.

The correlation between 1,25-$(OH)_2$-$VitD_3$ level and NRDS has been generally confirmed by studies so far. However, the associations of 1,25-$(OH)_2$-$VitD_3$ level with the development and prognosis of NRDS have been rarely reported. This study aims to investigate the associations of serum 1,25-$(OH)_2$-$VitD_3$ level in neonates with NRDS and the prognosis, so as to provide some references for the clinical treatment of NRDS.

MATERIALS AND METHODS

The clinical data of 145 NRDS children admitted to the neonatal ward of our hospital from April 2019 to June 2020 were retrospectively analyzed. The subjects included 76 boys and 69 girls, with a gestational age of 39.37 ± 1.54 weeks and body mass of 2.39 ± 0.76 kg. Based on NRDS severity, the children were assigned into a mild group (n = 82) and severe group (n = 63). Inclusion criterion: children conformed to the diagnostic criteria of NRDS.

Exclusion criteria were set as follows: children complicated with congenital diseases, deformity, heart failure, severe infection, anemia, pulmonary hemorrhage, or intracranial hemorrhage. This study was approved by the Ethics Committee of our hospital, and all the family members and guardians of the subjects signed the informed consent.

NRDS [11] was diagnosed based on the following criteria: clinical adverse symptoms, such as typical bronchial inflation sign in X-ray examination, gridding shadows or white lung NRDS signs appear within 24 hours after birth, clinically manifested as progressive dyspnea, moaning, shortness of breath, and inspiratory three depressions sign. NRDS severity was graded as follows [12]: Grade I: fine miliary ground-glass shadows and opacification of both lungs. Grade II: empty bronchial shadow beyond heart shadow in addition to miliary shadows. Grade III: blurred cardiac and septal margins in addition to the above shadows, and aerated bronchus sign. Grade IV: extensive white shadow (white lung), and severer aerated bronchus sign. Grade I and Grade II NRDS were mild, while Grade III and Grade IV NRDS were severe.

Peripheral venous blood samples (8 mL) were collected from the children on the day of admission or the next day before antibiotic was administered, and the serum sample was collected after centrifugation (3,000 rpm, 15 minutes), followed by determination of procalcitonin (PCT), C-reactive protein (CRP), serum bicarbonate, 1,25-$(OH)_2$-$VitD_3$, vitamin A, activin-A, Clara cell protein-16 (CC-16), interleukin-8 (IL-8), IL-6, IL-10, and IL-18 levels.

For the detection of 1,25-$(OH)_2$-$VitD_3$ the serum was immediately separated. Within 1 hour, the measurement was conducted in strict accordance with the instructions of corresponding enzyme-linked immunosorbent assay kit (Diasorin, USA). The absorbance was measured with SpectraMax iD3 microplate reader (Molecular Devices, USA) to calculate the level of 1,25-$(OH)_2$-$VitD_3$.

SPSS 23.0 software was utilized for statistical analysis. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared by *t*-test between groups. The numerical data were expressed as percentage (%), and compared by χ^2 between groups. A multivariate logistic regression model was employed to analyze the factors affecting NRDS severity in children. Spearman's method was adopted to evaluate the correlations of serum 1,25-$(OH)_2$-$VitD_3$ level with NRDS severity and other related blood test indices. $p < 0.05$ indicated statistically significant differences.

RESULTS

No statistically significant differences were observed in gender, body mass, age upon admission, twin pregnancy, mode of delivery, birth asphyxia, premature rupture of membranes, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol between the severe group and mild group ($p > 0.05$). The number of children with a gestational age < 39 weeks, spontaneous delivery, selective cesarean section, intrauterine distress, intrauterine infection, maternal hypertension, and maternal dyslipidemia were higher and the maternal age

Table 1. Univariate analysis results of NRDS severity in children.

Item	Severe group (n = 63)	Mild group (n = 82)	χ^2/t	p
Maternal age	29.37 ± 6.73	25.91 ± 5.24	3.481 [a]	0.001
Boy [n (%)]	32 (50.79%)	42 (51.23%)	0.003	0.959
Gestational age < 39 weeks	41 (65.08%)	39 (47.56%)	4.421	0.036
Body mass < 2,500 g	13 (20.63%)	11 (13.41%)	1.345	0.246
Age upon admission (hour)	3.38 ± 1.21	3.29 ± 1.43	0.401 [a]	0.689
Twin pregnancy	3 (4.76%)	4 (4.88%)	0.001	0.974
Mode of delivery				
Spontaneous delivery	15 (23.81%)	33 (40.24%)	4.345	0.037
Cesarean section	21 (33.33%)	20 (24.39%)	1.405	0.236
Selective cesarean section	27 (42.86%)	20 (24.39%)	5.546	0.019
Intrauterine distress	19 (30.16%)	11 (13.41%)	6.087	0.014
Birth asphyxia	21 (33.33%)	20 (24.39%)	1.405	0.236
Premature rupture of membranes	18 (28.57%)	13 (15.85%)	3.428	0.064
Placental abruption	26 (41.27%)	24 (29.27%)	2.271	0.132
Placenta previa	15 (23.81%)	19 (23.17%)	0.008	0.928
Amniotic fluid contamination	16 (25.40%)	12 (14.63%)	2.649	0.104
Meconium contamination	9 (14.29%)	11 (13.41%)	0.023	0.880
Severe pneumonia	22 (34.92%)	17 (20.73%)	3.648	0.056
Intrauterine infection	18 (28.57%)	9 (10.98%)	7.279	0.007
Maternal disease				
Diabetes	12 (10.05%)	10 (12.19%)	1.300	0.254
Hypertension	24 (38.10%)	31 (37.80%)	0.001	0.972
Gestational hypertension	27 (42.86%)	20 (24.39%)	5.546	0.019
Dyslipidemia	21 (33.33%)	13 (15.85%)	6.064	0.014
PCT (g/L)	6.57 ± 1.24	3.26 ± 0.14	23.999 [a]	< 0.001
CRP (mg/L)	19.21 ± 4.07	12.35 ± 3.96	10.216 [a]	< 0.001
Serum bicarbonate (mmol/L)	22.37 ± 7.63	25.46 ± 8.35	2.292 [a]	0.023
$1,25\text{-}(OH)_2\text{-}VitD_3$ (mmol/L)	35.46 ± 3.67	57.85 ± 6.31	20.600 [a]	< 0.001
Vitamin A (mmol/L)	39.87 ± 4.59	48.66 ± 5.38	10.384 [a]	< 0.001
Activin-A (pg/mL)	1,528.83 ± 27.36	773.82 ± 11.24	226.430 [a]	< 0.001
CC-16 (ng/L)	87.49 ± 9.34	62.56 ± 6.72	18.688 [a]	< 0.001
IL-6 (ng/L)	57.64 ± 6.35	57.57 ± 6.24	0.066 [a]	0.947
IL-8 (ng/L)	59.27 ± 7.09	108.65 ± 6.98	41.939 [a]	< 0.001
IL-18 (ng/L)	136.27 ± 7.09	88.59 ± 6.37	42.530 [a]	< 0.001
IL-10 (ng/L)	20.33 ± 2.98	19.02 ± 3.12	2.555 [a]	0.012

[a] indicates *t* value, while the others are χ^2 value.

was older in severe group than those in the mild group ($p < 0.05$). In terms of laboratory indices, PCT, CRP, activin-A, CC-16, IL-18, and IL-10 were significantly higher in the severe group than those in the mild group ($p < 0.05$), while serum bicarbonate, $1,25\text{-}(OH)_2\text{-}VitD_3$, vitamin A, and IL-8 were significantly lower in the severe group than those in the mild group ($p < 0.05$) (Table 1).

Multivariate logistic regression analysis was conducted with NRDS severity as the dependent variable (yes = 1, no = 0) and the indices with statistically significant differences in univariate analysis as the independent vari-

ables (Table 2). The results showed that gestational age < 39 weeks, lower 1,25-$(OH)_2$-$VitD_3$ and vitamin A levels, and higher activin-A and CC-16 levels were independent risk factors for severe NRDS ($p < 0.05$) (Table 3).

Table 2. Variable assignments for multivariate logistic regression analysis of NRDS severity in children.

Variable	Assignment
Severe NRDS	yes = 1, no = 0
Maternal age (years)	> 26 = 1, ≤ 26 = 0
Gestational age < 39 weeks	yes = 1, no = 0
Spontaneous delivery	no = 1, yes = 0
Selective cesarean section	yes = 1, no = 0
Intrauterine distress	yes = 1, no = 0
Intrauterine infection	yes = 1, no = 0
Gestational hypertension	yes = 1, no = 0
Dyslipidemia	yes = 1, no = 0
PCT (g/L)	> 4.5 = 1, ≤ 4.5 = 0
CRP (mg/L)	> 15 = 1, ≤ 15 = 0
Serum bicarbonate (mmol/L)	< 23.5 = 1, ≥ 23.5 = 0
1,25-$(OH)_2$-$VitD_3$ (mmol/L)	< 45 = 1, ≥ 45 = 0
Vitamin A (mmol/L)	< 40 = 1, ≥ 40 = 0
Activin-A (pg/mL)	> 900 = 1, ≤ 900 = 0
CC-16 (ng/L)	> 75 = 1, ≤ 75 = 0
IL-8 (ng/L)	< 75 = 1, ≥ 75 = 0
IL-18 (ng/L)	> 100 = 1, ≤ 100 = 0
IL-10 (ng/L)	> 20 = 1, ≤ 20 = 0

On the basis of factors affecting NRDS severity, a nomogram model was established to predict the probability of severe NRDS in children. By comparing the nomogram, it was found that the total score of the five influencing factors was 243.77 points, and the corresponding risk value of severe NRDS was 0.69, so the predicted probability of severe NRDS in children was 69% (Figure 1).

The model correctly predicted 75 cases of mild NRDS and 58 cases of severe NRDS, but incorrectly predicted 11 cases of mild NRDS and 7 cases of severe NRDS. Prediction accuracy of the model = (75 + 58)/(75 + 58 + 11 + 7) × 100% = 88.08% (Table 4). The C-index value of the model was 0.756 (95% CI: 0.684 - 0.853), and the receiver operating characteristic (ROC) curve suggested that the area under the ROC curve of the model was 0.785 (Figure 2).

The correlations of serum 1,25-$(OH)_2$-$VitD_3$ level in NRDS children with NRDS severity and other related blood test indices were analyzed using Spearman's and Pearson's methods. The results manifested that serum 1,25-$(OH)_2$-$VitD_3$ level was significantly negatively correlated with NRDS severity ($r = -0.287$, $p < 0.001$), activin-A ($r = -0.073$, $p < 0.001$), and CC-16 ($r = -0.098$, $p < 0.001$), but positively correlated with vitamin A ($r = 0.009$, $p < 0.001$) (Table 5).

Based on mortality status of NRDS children, 1,25-$(OH)_2$-$VitD_3$ level was divided into high- and low-level groups by the ROC curve. The greatest Yoden's index (1,25-$(OH)_2$-$VitD_3$ = 45 mmol/L, Youden's index = 0.391) was taken as the cutoff value. Survival analysis was conducted on NRDS children, and the results revealed that there were 78 cases with a high 1,25-$(OH)_2$-$VitD_3$ level (≥ 45 mmol/L) and 67 cases with a low 1,25-$(OH)_2$-$VitD_3$ level (< 45 mmol/L). At 72 hours after birth, 3 children died in 1,25-$(OH)_2$-$VitD_3$ high level group, while 9 died in 1,25-$(OH)_2$-$VitD_3$ low level group. The Kaplan-Meier curve showed that there was a significant difference in the mortality probability between 1,25-$(OH)_2$-$VitD_3$ high level group and 1,25-$(OH)_2$-$VitD_3$ low level group ($p < 0.05$) (Figure 3).

DISCUSSION

NRDS, a common acute and critical disease in clinical pediatrics, is primarily caused by progressive alveolar collapse due to pulmonary surfactant deficiency [13-15]. NRDS can induce progressive dyspnea, moaning, cyanosis, inspiratory three depressions sign and even respiratory failure within 12 hours after birth, and it is one of the biggest contributors to neonatal death [16]. The synthesis of pulmonary surfactant is affected by a variety of hormones and vitamin D is one of the steroids. As the major active form of vitamin D3, 1,25-$(OH)_2$-$VitD_3$ can participate in multiple biological reactions such as bone growth, absorption of trace elements, and immune regulation through binding to vitamin D receptors [17]. It has been reported that 1,25-$(OH)_2$-$VitD_3$ also exerts a certain regulatory effect on the respiratory system, and 1,25-$(OH)_2$-$VitD_3$ deficiency is prone to diseases such as repeated respiratory infections and bronchial asthma [18]. A recent study [19] has revealed that 1,25-$(OH)_2$-$VitD_3$ can facilitate the synthesis of pulmonary surfactant, and pulmonary surfactant insufficiency is the root cause of NRDS, indicating a close correlation between 1,25-$(OH)_2$-$VitD_3$ level and NRDS. The study by Hesse et al. [20] suggested that serum 1,25-$(OH)_2$-$VitD_3$ level in NRDS children was significantly lower than that in healthy neonates, and NRDS children had a higher rate of vitamin D deficiency. Grant et al. [21] found that vitamin D deficiency was an independent risk factor for NRDS and was closely associated with the grade and development of NRDS. Hence, it was speculated in this study that serum 1,25-$(OH)_2$-$VitD_3$ level may be implicated in the development of NRDS and affect the prognosis of children. In this study, 145 NRDS children were assigned into a mild group and a severe group according to NRDS severity. General data comparison between the two groups indicated that 1,25-$(OH)_2$-$VitD_3$ level was

Table 3. Multivariate analysis results of NRDS severity.

Variable	Regression coefficient	Standard error	Wald χ^2	*OR*	95% CI	p
Maternal age	1.210	0.741	2.667	3.354	0.002 - 4.536	0.536
Gestational age < 39 weeks	1.240	0.628	3.901	3.457	1.216 - 4.789	< 0.001
Spontaneous delivery	1.395	0.729	3.662	4.035	0.032 - 5.437	0.938
Selective cesarean section	1.473	0.861	2.928	4.364	0.049 - 5.564	0.157
Intrauterine distress	1.442	0.644	5.011	4.228	0.063 - 5.261	0.634
Intrauterine infection	1.548	0.831	3.472	4.704	0.082 - 5.875	0.453
Gestational hypertension	1.656	0.723	5.247	5.239	0.094 - 6.372	0.456
Dyslipidemia	1.567	0.591	7.033	4.794	0.135 - 5.848	0.945
PCT	1.756	0.761	5.326	5.791	0.639 - 6.765	0.526
CRP	1.569	0.598	6.888	4.804	0.648 - 5.257	0.335
Serum bicarbonate	-0.242	0.135	3.213	0.785	0.582 - 1.389	0.274
1,25-(OH)2-VitD3	-0.435	0.198	6.940	0.647	0.494 - 0.872	< 0.001
Vitamin A	-0.936	0.702	1.780	0.392	0.135 - 0.548	< 0.001
Activin-A	1.979	0.696	8.084	7.235	2.936 - 10.587	< 0.001
CC-16	1.894	0.854	4.921	6.649	2.294 - 11.645	< 0.001
IL-8 (ng/L)	-1.121	0.728	2.371	0.326	0.202 - 1.436	0.215
IL-18 (ng/L)	1.807	0.699	6.683	6.092	0.816 - 8.289	0.084
IL-10 (ng/L)	1.877	0.729	6.630	6.534	0.939 - 8.906	0.056

Table 4. Confusion matrix for the model predicting severe NRDS in children.

Severity	n	Predicted cases		Reliability (%)		Accuracy (%)	
		Mild NRDS	Severe NRDS	Mild NRDS	Severe NRDS	Mild NRDS	Severe NRDS
Mild NRDS	82	75	7	91.46	8.54	80.65	9.46
Severe NRDS	63	11	58	17.46	92.06	11.83	78.38

Table 5. Results of correlation analysis between 1,25-$(OH)_2$-$VitD_3$ and NRDS severity.

Item	1,25-(OH)2-VitD3	
	r	p
NRDS severity	-0.287	< 0.001
PCT	-0.207	0.962
CRP	-0.234	0.315
Serum bicarbonate	0.426	0.249
Vitamin A	0.009	< 0.001
Activin-A	-0.073	< 0.001
CC-16	-0.098	< 0.001
IL-8 (ng/L)	0.326	0.052
IL-18 (ng/L)	-0.034	0.378
IL-10 (ng/L)	-0.561	0.243

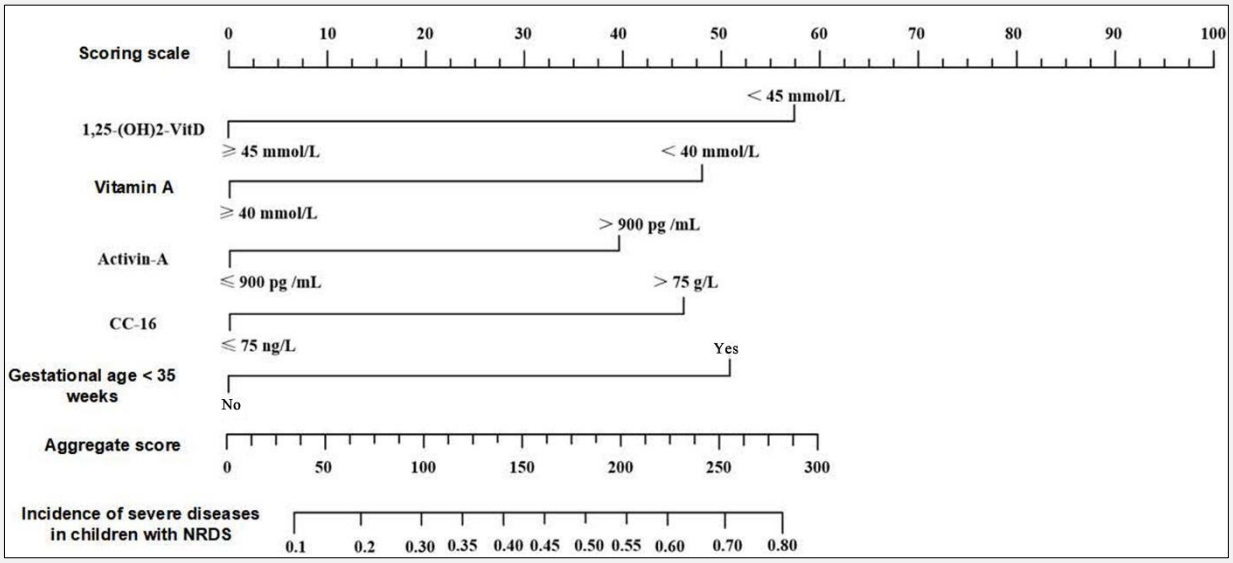

Figure 1. Nomogram model for predicting severe NRDS in children. 1,25-$(OH)_2$-$VitD_3$, vitamin A, activin-A, CC-16, and gestational age were incorporated into the model to predict the severity of NRDS, with high clinical value.

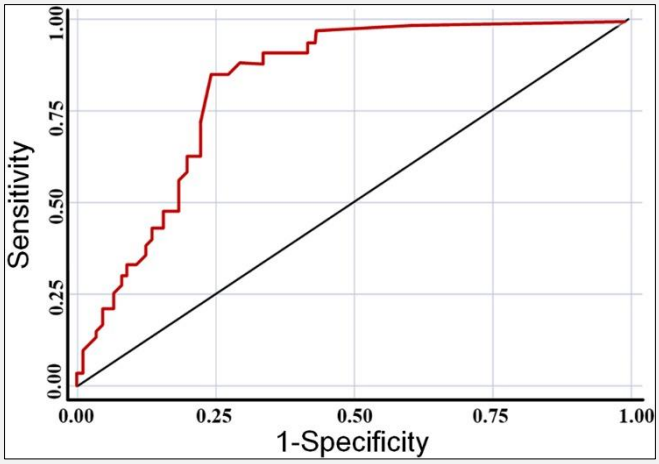

Figure 2. ROC curve of the model predicting NRDS severity in children.

significantly lower in the severe group than that in the mild group. The results of multivariate logistic regression analysis confirmed that low 1,25-$(OH)_2$-$VitD_3$ level was an independent risk factor for severe NRDS. Moreover, four other independent influencing factors, gestational age < 39 weeks, vitamin A, activin-A, and CC-16, were identified. According to a previous study [22], gestational age is the major factor affecting the incidence of NRDS, and the smaller the gestational age, the higher the incidence rate. Vitamin A can facilitate

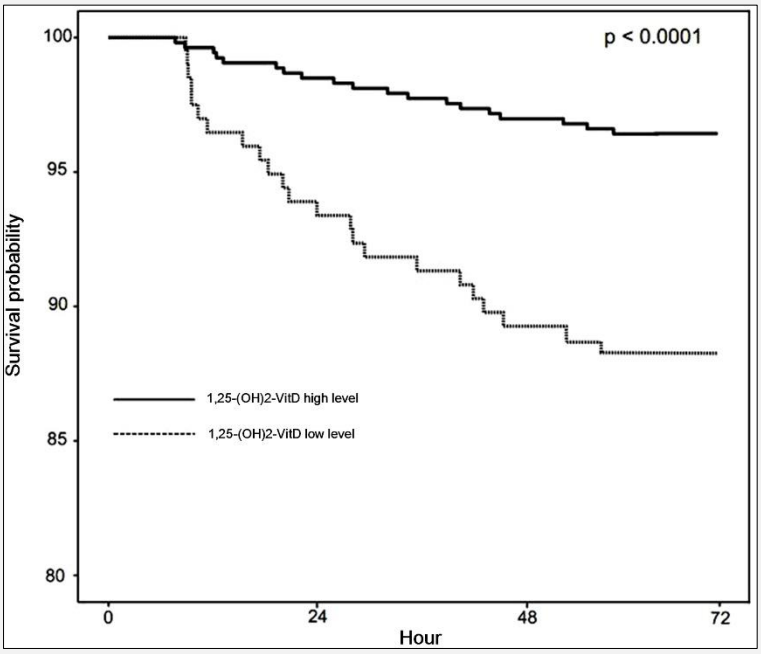

Figure 3. Correlation between 1,25-$(OH)_2$-$VitD_3$ and survival rate of NRDS children.

the synthesis and release of pulmonary surfactant, and is transmitted to the fetus only in the third trimester of pregnancy. Hence, vitamin A deficiency may be induced in the cases of premature delivery, thus increasing the probability of NRDS. Elfarargy researched and found that [23] vitamin A was associated with NRDS severity, and neonates with Grade III and IV NRDS had a lower serum vitamin A level than those with Grade I and II NRDS. Activin-A, a pleiotropic cytokine, is closely associated with the degree of inflammatory response to respiratory diseases. The study by Kim et al. [24] revealed that serum activin-A level variation was associated with the severity and prognosis of NRDS, and effective monitoring of activin-A level can help evaluate the progression of patients' disease and provide a basis for the treatment and prognosis prediction. CC-16, a functional protein secreted by Clara cells, exerts vital regulatory effects on respiratory tract injury and inflammatory response. It has been revealed [25] that serum CC-16 level is a good indicator for assessing the severity and prognosis of NRDS, and the higher the serum CC-16 level, the severer the patient's condition and the worse the prognosis. In this study, the correlations of serum 1,25-$(OH)_2$-$VitD_3$ level in NRDS children with NRDS severity and other related blood test indices were analyzed using Spearman's and Pearson's methods. The results manifested that serum 1,25-$(OH)_2$-$VitD_3$ level was significantly negatively correlated with NRDS severity, activin-A, and CC-16, but positively correlated with vitamin A, indicating that 1,25-$(OH)_2$-$VitD_3$ can be implicated in the development of NRDS and affect the prognosis of NRDS children. A nomogram model for predicting NRDS severity in children was established based on the independent influencing factors, whose predictive value was confirmed by ROC curve analysis.

In conclusion, 1,25-$(OH)_2$-$VitD_3$ is an independent influencing factor for the severity of NRDS and may affect the prognosis of NRDS children. Attention should be paid to the variation of 1,25-$(OH)_2$-$VitD_3$ level in NRDS children during clinical treatment. The degree of NRDS can be assessed by measuring the serum 1,25-$(OH)_2$-$VitD_3$ level to make timely adjustments to the treatment regimen, thereby improving the prognosis. Regardless, this study has limitations. This is a single-center retrospective study with a small sample size, so the results may be biased. The findings herein need to be verified by prospective studies with larger sample sizes.

Declaration of Interest:
None to declare.

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