

Association of umbilical cord blood miR-375 with neonatal respiratory distress syndrome and adverse neonatal outcomes in premature infants

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Background: Neonatal respiratory distress syndrome (NRDS) is a common respiratory disorder occurring in premature infants, and some microRNAs (miRNAs) have been demonstrated to play critical roles in NRDS progression. This study aimed to measure relative expression of miR-375 in infants with NRDS, and further evaluate the clinical significance of miR-375 in predicting the onset and clinical prognosis of NRDS in infants. **Methods:** This study collected umbilical cord blood from 180 premature neonates, including 90 neonates with NRDS and 90 non-NRDS neonates. Quantitative real-time PCR was used to detect relative expression level of miR-375. The diagnostic value of miR-375 in screening NRDS neonates from control neonates and its predictive accuracy for clinical prognosis were evaluated by receiver operating characteristic analysis. The relationship of miR-475 with disease onset and clinical outcomes in NRDS infants was assessed by univariate and multivariate logistic regression analyses. **Results:** Relative miR-375 expression was upregulated in NRDS neonates, and high levels of miR-375 were observed in NRDS grade III-IV cases compared to those early-stage neonates. miR-375 had relatively high diagnostic accuracy to screen NRDS neonates and was independently associated with NRDS onset in infants. Moreover, relative miR-375 expression was upregulated in NRDS neonates with poor prognosis and could independently predict the clinical outcomes of NRDS neonates with considerable predictive accuracy. **Conclusion:** Umbilical cord serum miR-375 is elevated and associated with NRDS onset and clinical outcomes in NRDS neonates. Thus, miR-375 may serve as a biomarker for the diagnosis and prognosis of infants with NRDS.

Keywords: microRNA-375; diagnosis; prognosis; neonatal respiratory distress syndrome; premature; prediction

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Abbreviations: AMI, acute myocardial infarction; AUC, area under the curve; CI, confidence interval; HER-2, human epidermal growth factor receptor 2; MTC, medullary thyroid carcinoma; miRNAs, microRNAs; mRNAs, messenger RNAs; OR, odds ratio; NRDS, neonatal respiratory distress syndrome; ROC, receiver operating characteristic; PS, pulmonary surfactant; qRT-PCR, quantitative real-time PCR; YAP1, yes-associated protein 1

INTRODUCTION

Neonatal respiratory distress syndrome (NRDS), also known as neonatal pulmonary hyaline, is where adverse symptoms, such as progressive dyspnea and res-

piratory failure, develop soon after birth in a newborn baby. It is mainly caused by the absence of pulmonary surfactant (PS), leading to a progressive alveolar collapse (Huo *et al.*, 2020). PS refers to a complex lipoprotein secreted by lung alveolar type-II epithelial cells and distributed on the surface of the molecular layer of alveolar fluid, which can reduce alveolar surface tension, can maintain relative stability of the volume of both large and small alveoli, and can prevent fluid in alveolar capillaries from filtering out into the alveoli (Wang *et al.*, 2021; Zhuo *et al.*, 2021). NRDS newborns are mostly premature (Kaya *et al.*, 2017; Liszewski *et al.*, 2017). Their clinical signs (such as dyspnea, moaning, cyanosis, and the inspiratory triple concave sign) become very obvious 6 hours after birth, and they even show symptoms of irregular breathing, apnea, and respiratory failure (Li *et al.*, 2020d). Their incidence is correlated with gestational age, which increased with smaller size for gestational age (Sweet *et al.*, 2013). Besides, their mortality is weight related, with a higher case fatality rate for lower body weight (Qian *et al.*, 2010). NRDS is a self-limited disease, and those who can survive more than three days have increased lung maturity and greater hope of recovery (Han & Mallampalli, 2015). However, a number of infants have complicated pneumonia (Zheng & Sun, 2020), which continues to worsen until infection control improves. Infants with severe disease mostly die within three days, with the highest case fatality rate occurring on the second day after birth. Therefore, early prediction of the risk of NRDS occurrence and early preparation for prevention and treatment of NRDS are important to improve the prognosis of premature infants.

MicroRNA (miRNAs) are a class of small non-coding RNAs that can modulate gene expression, mainly through translational inhibition or degradation of messenger RNAs (mRNA). miRNAs are stably present in the body fluid environment. miRNAs with aberrant expression levels in different pathological conditions have important indicative roles for disease initiation and progression, and are the basis for the development of more and effective clinical biomarkers (Chen *et al.*, 2019). An important previous report has found that miR-375 can regulate PS secretion in alveolar type-II epithelial cells by affecting cytoskeleton reorganization (Zhang *et al.*, 2010). In addition, miR-375 also has the ability to regulate trans-differentiation of rat alveolar epithelial cells (Wang *et al.*, 2013). Given that PS deficiency causes NRDS, this study is reasonable to speculate that there is a link between miR-375 levels and the disease occurrence of NRDS.

Therefore, the purpose of this study was to evaluate the predictive value of miR-375 in the development of NRDS and analyze the correlation between miR-375 levels and neonatal clinical outcomes based on the analysis of relative miR-375 levels in umbilical cord blood of premature infants. This study will provide new biomarkers for early diagnosis and disease prognosis of NRDS, thereby improving the prevention, diagnosis, and treatment of NRDS.

MATERIALS AND METHODS

Premature infants

This study was approved by the Ethics Committee of Changyi Maternal And Child Care Hospital. A total of 180 premature infants born at Changyi Maternal And Child Care Hospital, between 2016 and 2020, were included in this study, including 90 newborns with NRDS and 90 control newborns without NRDS. The diagnosis of NRDS was mainly judged based on clinical symptoms and imaging findings, including a respiratory rate of more than 60 breaths/min, grunting, dyspnea, shortness of breath, cyanosis, intercostal, subcostal or suprasternal retraction, and bronchial congestion, reticular shadow or white lung found by X-ray. Inclusion criteria were as follows: (a) all participants were premature infants with a gestational age of 25–36 weeks; (b) newborns with NRDS fulfilled the diagnostic criteria for NRDS and (c) control newborns did not present any clinical signs of NRDS. Exclusion criteria were: (a) newborn was term infant and/or (b) the newborn had other conditions that cause difficult breathing, including congenital malformations of the heart and respiratory tract, intrauterine infectious pneumonia, and meconium aspiration. Identification of group B streptococcal infectious pneumonia (which is the most common intrauterine infectious pneumonia) was as follows: pregnant women had a history of premature rupture of amnion, intrauterine infection or odor of amniotic fluid, lung X-ray changes had different degrees of fusion trend, the course of disease is different from NRDS (newborns with the above manifestations

were excluded from this study as they had the potential to have group B hemolytic streptococci), and group B hemolytic streptococci are found in blood culture of pregnant mothers and children, cervical and pharyngeal swabs of pregnant mothers. The guardians of all participating newborns signed informed consent forms. The severity of NRDS was defined according to X-ray grading (Sawires *et al.*, 2015). Grade I: the field brightness of both lungs was markedly reduced, and evenly scattered fine particles and reticular shadowing were visible; Grade II: in addition to grade I change aggravation, bronchial inflation signs appeared, extending into the mid outer band of the lung field; Grade III: the lesions worsened, lung field transillumination was more reduced, and the heart margin and diaphragmatic surface were blurred; and Grade IV: the whole lung field showed white lung, and the bronchial inflation sign was more significant. Mild NRDS included grade I and II (56 cases), and severe NRDS included grade III and IV (34 cases).

Umbilical cord blood collection and serum preparation

Umbilical cord blood was collected rapidly when the umbilical cord was interrupted from the end of the maternal parent. Then, serum samples were separated from 3ml of umbilical cord blood by centrifugation at low-temperature and stored at -80°C for further use.

RNA extraction

Total RNA was extracted from the serum of NRDS newborns and control newborns using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The purity and concentration of the obtained RNA were evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Inc.) following the manufacturers' instructions. When the optical density (OD) ratio of A260/280 was close to 2.0, RNA was used for further analysis.

Quantitative real-time PCR (qRT-PCR)

The obtained RNA was reverse transcribed into cDNA using the PrimeScript RT Reagent Kit (TaKaRa, Japan). SYBR green I Master Mix kit (Invitrogen,

Table 1. Baseline characteristics of the included infants

Variables	Controls (n=90)	NRDS (n=90)	P value
Gestational age (weeks)	30.94±3.36	30.82±3.45	0.858
Gender (males, n)	61 (67.8%)	63 (70%)	0.747
Birth weight (g)	1933.07±381.01	1922.64±381.05	0.852
Maternal GDM	7 (7.8%)	17 (18.9%)	0.028
Delivery method			
Vaginal	56 (62.2%)	51 (56.7%)	0.448
Caesarean	34 (37.8%)	39 (43.3%)	
Apgar score			
1 min	7.22±1.35	5.02±1.69	<0.001
5 min	8.98±0.99	6.47±1.56	< 0.001
NRDS grades			
I	–	24 (26.7%)	
II	–	32 (35.5%)	–
III	–	25 (27.8%)	
IV	–	9 (10.0%)	

GDM, gestational diabetes mellitus; NRDS, neonatal respiratory distress syndrome.

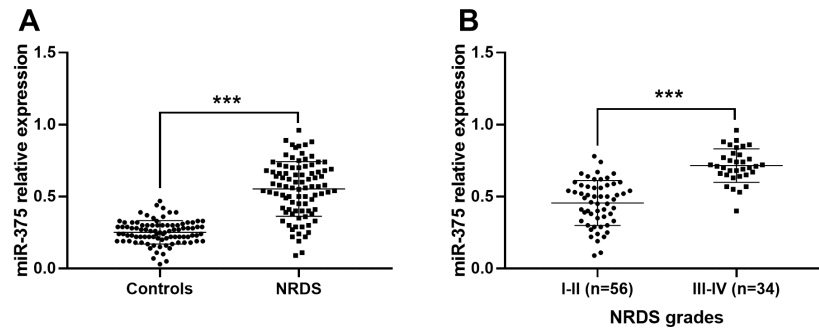


Figure 1. Relative expression of miR-375 in NRDS and control groups.

(A) Relative miR-375 expression was higher in NRDS group than that in control group. (B) Relative miR-375 expression was higher in premature infants with NRDS grade III-IV compared with that in neonates with NRDS grade I-II. *** $P < 0.001$ vs. Controls or neonates with NRDS grade I-II. NRDS, neonatal respiratory distress syndrome.

Carlsbad, CA, USA) on the 7900HT fast real-time PCR system (Applied Biosystems, San Francisco, CA, United States) was used for qRT-PCR, which was performed to measure the relative expression of miR-375. The following thermo cycling conditions were used for the qPCR: 95°C for 10 min, followed by 40 cycles of 95°C for 20 s and 60°C for 15 s, 72°C for 20 s. The cel-miR-39-3p was used as the internal reference of relative miR-375 expression. The primer sequences were: miR-375 forward, 5'-GCCGAGTTTGTTTCGTTTCGGC-3' and reverse 5'-CTCAACTGGTGTCTGTGGA-3'; cel-miR-39-3p forward, 5'-UCACCGGGUGUAAAUCAGC-UUG-3' and reverse, 5'-AACGCTTCACGAATTTCGT-3'. Relative miR-375 expression was calculated by $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). All procedures were performed according to the manufacturers' instructions.

Record of neonatal outcomes

Clinical outcome at neonatal discharge was recorded and divided into two types. (a) Improved or cured (favourable): after treatment, neonatal vital signs were stable, neonatal respiratory symptoms and signs were significantly reduced or disappeared, neonatal chest radiograph manifestations were basically normal, and the newborn could be separated from ventilator support and oxygen therapy. (b) Give up or die (poor): neonatal treatment effects were unsatisfactory, neonatal vital signs were unstable, newborns could not be separated from ventilator support, newborns combined with severe complications, and/or family members requested abandonment of treatment resulting in neonatal discharge or disappearance of vital signs, which in turn declared clinical death, because of factors such as economic conditions or prognosis.

Statistical analysis

Data analysis results were shown as mean \pm SD or number (percentage). All analyses were performed by SPSS 22.0 (IBM Corp.) and GraphPad Prism 7.0 software (GraphPad Software, Inc.). Comparisons between two groups of measurement data were performed using Mann-Whitney U-test. Chi-square test was used to analyze differences between categorical variables. Receiver operating characteristic (ROC) analysis was performed to analyze the ability of miR-375 to discriminate between NRDS and control groups, and to discriminate between NRDS neonates with favourable and poor clinical outcomes. Logistic regression analy-

sis was used to evaluate whether miR-375 could predict NRDS onset in premature infants and to predict clinical outcomes in NRDS neonates. $P < 0.05$ indicated a statistically significant difference.

RESULTS

Baseline characteristics of the included infants

As shown in Table 1, there were no significant differences between the control and NRDS groups in gestational age, gender, birth weight, and delivery method (all $P > 0.05$). Additionally, the NRDS group had more premature infants with maternal GDM ($P = 0.028$), and lower Apgar scores at 1 and 5 min (all $P < 0.001$) than the control group. The number of premature infants with NRDS grade I-IV was 24, 32, 25, and 9, respectively.

Differentially expressed miR-375 in the newborns between NRDS and control groups

The relative expression of miR-375 was significantly increased in the NRDS group compared with that in the control group ($P < 0.001$, Fig. 1A). The Fig. 1B revealed that miR-375 relative expression in neonates with NRDS grade III-IV was significantly higher than that in neonates with NRDS grade I-II ($P < 0.001$), suggesting an association between miR-375 relative expression and NRDS severity.

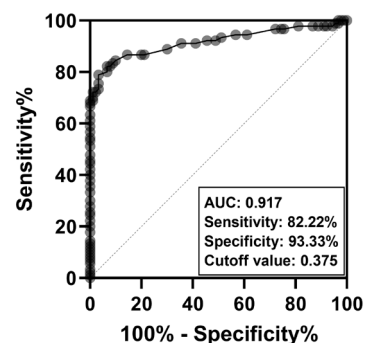


Figure 2. ROC analysis results indicated that miR-375 had a high diagnostic value to screen NRDS neonates from control neonates with an AUC of 0.917.

AUC, area under the ROC curve; ROC, receiver operating characteristic; NRDS, neonatal respiratory distress syndrome.

Table 2. Risk factor analysis to predict NRDS onset in premature infants using logistic analysis

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Gestational age	1.223	0.871–1.669	0.189	–	–	–
Gender	1.189	0.764–1.596	0.376	–	–	–
Birth weight	1.372	0.940–1.886	0.112	–	–	–
Maternal GDM	1.469	1.109–1.913	0.038	1.412	0.998–1.907	0.059
Delivery method	1.171	0.913–1.447	0.166	–	–	–
Apgar score–1 min	1.781	1.236–2.298	0.042	1.567	0.997–2.184	0.052
Apgar score–5 min	2.033	1.437–2.739	0.024	1.975	1.413–2.660	0.032
miR–375	2.408	1.601–3.228	0.003	2.223	1.579–2.995	0.009

GDM, gestational diabetes mellitus; NRDS, neonatal respiratory distress syndrome; OR, odds ratio; CI, confidence interval.

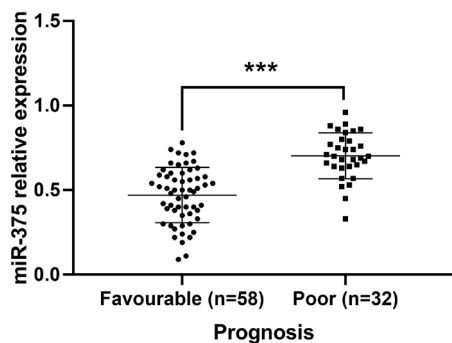


Figure 3. Relative miR-375 expression was higher in NRDS neonates with poor prognosis than that in NRDS neonates with favourable prognosis. *** $P < 0.001$ vs. NRDS neonates with favourable prognosis. NRDS, neonatal respiratory distress syndrome.

Early diagnostic performance of miR-375 to predict NRDS onset in premature infants

The results of ROC analysis shown in Fig. 2 demonstrated that miR-375 had high diagnostic accuracy in distinguishing NRDS neonates from controls, with an area under the ROC curve (AUC) of 0.917. The results of the logistic analysis of risk factors predicting NRDS onset in premature infants were presented in Table 2. Univariate logistic analysis results demonstrated that maternal GDM, Apgar score-1 min, Apgar score-5 min and

miR-375 were related to the onset of NRDS in premature infants. Significant baseline variables from univariate analysis were included in the multivariate analysis. Multivariate analysis results demonstrated that Apgar score-5 min [odds ratio (OR)=1.975, 95% confidence interval (CI)=1.413–2.660, $P=0.032$] and miR-375 (OR=2.223, 95% CI=1.579–2.995, $P=0.009$) could independently predict the onset of NRDS in premature infants.

Aberrantly expressed miR-375 in NRDS newborns with different clinical outcomes

As presented in Fig. 3, miR-375 relative expression was significantly increased in NRDS neonates with poor clinical outcomes compared to that in NRDS neonates with favourable clinical outcomes ($P < 0.001$).

Predictive value of miR-375 for the clinical outcomes of NRDS newborns

Logistic analysis was performed to analyze the risk factors for predicting the clinical outcomes of NRDS neonates, and the results were shown in Table 3. Apgar score-5 min, NRDS grades and miR-375 were found to be associated with the clinical outcomes of NRDS newborns using univariate logistic analysis. Significant variables from univariate analysis were then included in the multivariate analysis. Further multivariate logistic analysis results indicated that Apgar score-5 min (OR=1.373, 95% CI=1.087–1.734, $P=0.042$), NRDS grades (OR=1.976, 95% CI=1.323–2.616, $P=0.013$) and miR-375 (OR=2.074, 95% CI=1.467–2.728, $P=0.002$)

Table 3. Risk factor analysis to predict clinical outcomes of NRDS neonates using logistic analysis

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Gestational age	1.212	0.784–1.773	0.335	–	–	–
Gender	1.130	0.716–1.579	0.467	–	–	–
Birth weight	1.278	0.843–1.763	0.128	–	–	–
Maternal GDM	1.396	0.912–1.561	0.111	–	–	–
Delivery method	1.223	0.763–1.698	0.306	–	–	–
Apgar score – 1 min	1.389	0.982–1.561	0.060	–	–	–
Apgar score – 5 min	1.406	1.121–1.884	0.039	1.373	1.087–1.734	0.042
NRDS grades	1.983	1.379–2.678	0.009	1.976	1.323–2.616	0.013
miR–375	2.108	1.566–2.874	< 0.001	2.074	1.467–2.728	0.002

GDM, gestational diabetes mellitus; NRDS, neonatal respiratory distress syndrome; OR, odds ratio; CI, confidence interval.

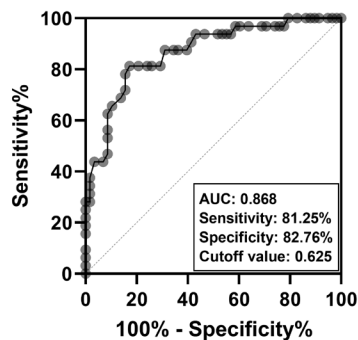


Figure 4. ROC analysis results showed that miR-137 had ability to differentiate between NRDS newborns with favourable and poor prognosis (AUC=0.868).

AUC, area under the ROC curve; ROC, receiver operating characteristic; NRDS, neonatal respiratory distress syndrome.

could independently predict the clinical outcomes of premature infants with NRDS. Figure 4 indicated that miR-137 had a high ability in differentiating NRDS neonates with favourable and poor clinical outcomes with an AUC of 0.868. At a cutoff value of 0.625, the sensitivity and specificity were 81.25% and 82.76%, respectively.

DISCUSSION

Correlation studies between miRNAs and diseases, including NRDS, have emerged but are less well studied. For example, miR-4319 is reduced in thyroid cancer tissues and cells, and suppresses the development of thyroid cancer (Bian, 2020). miR-25-3p can mitigate myocardial infarction (Peng *et al.*, 2020). Zhang and others (Zhang *et al.*, 2021) have found that miR-296-5p is increased in premature infants with NRDS and its increase can inhibit the secretion of PS in pulmonary epithelial cells. A study by Song and others (Song *et al.*, 2018) has shown that miR-216a-3p downregulation can cause differentiation of bone marrow mesenchymal stem cells into type-II alveolar epithelial cells, thus attenuating NRDS. This study found that relative miR-375 expression was markedly upregulated in NRDS newborns and NRDS newborns with advanced NRDS grades. In addition, miR-375 has been shown to exert a role in other diseases, such as nasopharyngeal carcinoma (Jia-Yuan *et al.*, 2020) and inflammatory bowel disease (Wu *et al.*, 2019). Thus, miR-375 may be correlated with the progression and disease severity of premature infants with NRDS.

Confirmation of dependable biomarkers with high sensitivity and specificity is of significant clinical value for early detection and treatment outcome prediction in NRDS. Numerous studies have indicated that miRNAs serve as promising biomarkers for various diseases. For instance, miR-1204 has been shown to serve as a diagnostic and prognostic biomarker in breast cancer (Han *et al.*, 2020). Circulating miR-1 is found to be a new biomarker for the diagnosis and prognostic prediction of acute myocardial infarction (AMI) (Liu *et al.*, 2020). miR-21 and miR-210 can function as novel biomarkers for colorectal cancer diagnosis and prognosis (Li *et al.*, 2020a).

Considering the high expression of miR-375 in NRDS newborns, especially those with higher NRDS grades, we further analyzed its clinical function in NRDS. Currently, no study has reported the role of miRNAs for predicting

the occurrence as well as the prognosis of NRDS. However, the clinical value of miRNAs has been reported in lung diseases and/or respiratory diseases. For instance, serum miR-1228-3p and miR-181a-5p can function as diagnostic and prognostic biomarkers for non-small cell lung cancer (Xue *et al.*, 2020). miR-125b can predict the risk and prognosis of acute respiratory distress syndrome in sepsis patients (Li *et al.*, 2020c). This study used ROC analysis and logistic regression analysis to investigate the ability of miR-375 to predict NRDS onset and NRDS prognosis in premature infants. The results indicated that miR-375 had a high diagnostic value for screening NRDS neonates from control neonates and was independently correlated with the onset of NRDS. Thus, miR-375 may serve as an independent biomarker in predicting NRDS onset in premature infants. Then, relative miR-375 expression was upregulated in NRDS newborns who had poor clinical outcomes and was an independent biomarker for predicting the clinical outcome of NRDS neonates. In addition, miR-375 has been reported to function as a biomarker for other diseases. For example, miR-375 can serve as a diagnostic biomarker for AMI (Ali Sheikh, 2020) and a prognostic biomarker for small cell lung cancer (Li *et al.*, 2020b). In addition, it can be used as a diagnostic and prognostic biomarker for medullary thyroid carcinoma (MTC) (Censi *et al.*, 2021). Thus, miR-375 may be a biomarker for the diagnosis and clinical prognosis of NRDS.

A study has revealed the inhibitory effect of miR-375 on PS secretion (Zhang *et al.*, 2010). Additionally, miR-375 is found to be associated with vitamin D, which is a well-documented risk factor for the onset of NRDS (Provvisiero *et al.*, 2019). The above studies may illustrate the cause of aberrant expression of miR-375 in NRDS and its role in predicting the occurrence of NRDS. In addition, miR-375 has the ability to regulate lung epithelial cell differentiation and proliferation (Cheng *et al.*, 2017), and it also has a significant effect on the activity of pulmonary microvascular endothelial cells (An *et al.*, 2020). The above studies illustrate that there is also a potential link between miR-375 and the disease course after NRDS disease onset, possibly explaining the important finding that miR-375 predicts clinical prognosis of NRDS neonates. In addition, it is worth noting that maternal GDM was found to be associated with NRDS onset in infants by logistic analysis, which is consistent with a previous study (Li *et al.*, 2019). Thus, impaired blood glucose during pregnancy should be diagnosed in time.

However, there were some limitations. First, the study sample was small, and a large sample is needed in future studies. Second, the mechanism by which miR-375 was involved in NRDS progression was not investigated. Thus, future studies on miR-375 should include mechanistic studies on the involvement of miR-375 in the progression of NRDS, in addition to further validating its clinical significance and promoting its clinical translational applications. It has been reported that miR-375 plays a role in asthma by targeting yes-associated protein 1 (YAP1) (Zhao *et al.*, 2020). Additionally, miR-375 can modulate human non-small cell carcinoma cells function via targeting human epidermal growth factor receptor 2 (HER-2) (Cheng *et al.*, 2017). Thus, the mechanism may be that miR-375 plays a role in NRDS by targeting YAP1 or HER-2, which needs to be studied in a future study. Notably, clinically administering glucocorticoids to mothers at risk of preterm delivery can induce lung maturation in preterm fetuses and prevent the development of respiratory distress syndrome. A study has assessed

the effect of prenatal dexamethasone (a type of glucocorticoids) administration on the miRNA expression profile in rat lung tissues (Yu *et al.*, 2016). Thus, we will study how prenatal steroid therapy affects the expression of miR-375 in the umbilical cord blood of human neonates in our future studies.

In conclusion, the findings of this study indicate that umbilical cord serum miR-375 is elevated in premature infants with NRDS. High miR-375 has relatively high diagnostic accuracy to screen NRDS neonates and predictive value for the clinical outcomes of premature NRDS neonates. Thus, our study data may provide a novel target to develop the diagnosis, prognosis, and therapy of NRDS.

Declaration

Ethics approval and consent to participate. The experimental procedures were all in accordance with the guideline of the Ethics Committee of Changyi Maternal And Child Care Hospital and has approved by the Ethics Committee of Changyi Maternal And Child Care Hospital. This study complies with the Declaration of Helsinki.

A signed written informed consent was obtained from each patient.

Consent for publication

Written informed consent for publication was obtained from each participant.

Availability of data and materials. The data used and analyzed can be obtained from the corresponding author under a reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. YD carried out the research design and conception; RZ analyzed and interpreted the data regarding; XJ performed the examination of sample; YD and RZ contributed essential reagents or tools; All authors wrote and revised the manuscript. All authors read and approved the final manuscript.

REFERENCES

Ali Sheikh MS (2020) Overexpression of miR-375 protects cardiomyocyte injury following hypoxic-reoxygenation injury. *Oxid Med Cell Longev* **2020**: 7164069. <https://doi.org/10.1155/2020/7164069>

An Y, Liu Z, Ding H, Lv Q, Fan H, Hou S, Cai W, Liu S (2020) MiR-375-3p regulates rat pulmonary microvascular endothelial cell activity by targeting Notch1 during hypoxia. *J Int Med Res* **48**: 300060520926851. <https://doi.org/10.1177/0300060520926851>

Bian S (2020) miR-4319 inhibited the development of thyroid cancer by modulating FUS-stabilized SMURF1. *J Cell Biochem* **121**: 174-182. <https://doi.org/10.1002/jcb.29026>

Censi S, Bertazza L, Piva I, Manso J, Benna C, Iacobone M, Mondin A, Plebani M, Faggian D, Galuppini F, Pennelli G, Barollo S, Mian C (2021) Serum miR-375 for diagnostic and prognostic purposes in medullary thyroid carcinoma. *Front Endocrinol (Lausanne)* **12**: 647369. <https://doi.org/10.3389/fendo.2021.647369>

Chen L, Heikkinen L, Wang C, Yang Y, Sun H, Wong (2019) Trends in the development of miRNA bioinformatics tools. *Brief Bioinform* **20**: 1836-1852. <https://doi.org/10.1093/bib/bby054>

Cheng L, Zhan B, Luo P, Wang B (2017) miRNA375 regulates the cell survival and apoptosis of human nonsmall cell carcinoma by targeting HER2. *Mol Med Rep* **15**: 1387-1392. <https://doi.org/10.3892/mmr.2017.6112>

Han S, Li P, Wang D, Yan H (2020) Dysregulation of serum miR-1204 and its potential as a biomarker for the diagnosis and prognosis of breast cancer. *Rev Assoc Med Bras (1992)* **66**: 732-736. <https://doi.org/10.1590/1806-9282.66.6.732>

Han S, Mallampalli RK (2015) The role of surfactant in lung disease and host defense against pulmonary infections. *Ann Am Thorac Soc* **12**: 765-774. <https://doi.org/10.1513/AnnalsATS.201411-507FR>

Huo MY, Mei H, Zhang YH, Liu CZ, Hu YN, Song D (2020) [Efficacy and safety of less invasive surfactant administration in the treatment of neonatal respiratory distress syndrome: a Meta analysis]. *Zhongguo Dang Dai Er Ke Za Zhi* **22**: 721-727 (in Chinese)

Jia-Yuan X, Wei S, Fang-Fang L, Zhi-Jian D, Long-He C, Sen L (2020) miR-375 Inhibits the proliferation and invasion of nasopharyngeal carcinoma cells by suppressing PDK1. *Biomed Res Int* **2020**: 9704245. <https://doi.org/10.1155/2020/9704245>

Kaya G, Sivasli E, Oztuzcu S, Melekoglu NA, Ozkara E, Sarikabadayi U, Demiryurek AT (2017) Association of Rho-kinase gene polymorphisms with respiratory distress syndrome in preterm neonates. *Pediatr Neonatol* **58**: 36-42. <https://doi.org/10.1016/j.pedneo.2015.12.006>

Li G, Wang Q, Li Z, Shen Y (2020a) Serum miR-21 and miR-210 as promising non-invasive biomarkers for the diagnosis and prognosis of colorectal cancer. *Rev Esp Enferm Dig* **112**: 832-837. <https://doi.org/10.17235/reed.2020.6801/2019>

Li M, Shan W, Hong B, Zou J, Li H, Han D, Zhang Y, Li L, Li D, Lin W (2020b) Circulating miR-92b and miR-375 for monitoring the chemoresistance and prognosis of small cell lung cancer. *Sci Rep* **10**: 12705. <https://doi.org/10.1038/s41598-020-69615-6>

Li S, Zhao D, Cui J, Wang L, Ma X, Li Y (2020c) Correlation of microRNA-125a/b with acute respiratory distress syndrome risk and prognosis in sepsis patients. *J Clin Lab Anal* **34**: e23098. <https://doi.org/10.1002/jcla.23098>

Li SN, Li L, Li CL, Zhou SP, Lu WC (2020d) The safety and effectiveness of heated humidified high-flow nasal cannula as an initial ventilation method in the treatment of neonatal respiratory distress syndrome: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)* **99**: e23243. <https://doi.org/10.1097/MD.00000000000023243>

Li Y, Wang W, Zhang D (2019) Maternal diabetes mellitus and risk of neonatal respiratory distress syndrome: a meta-analysis. *Acta Diabetol* **56**: 729-740. <https://doi.org/10.1007/s00592-019-01327-4>

Liszewski MC, Stanescu AL, Phillips GS, Lee EY (2017) Respiratory distress in neonates: Underlying causes and current imaging assessment. *Radiol Clin North Am* **55**: 629-644. <https://doi.org/10.1016/j.rcl.2017.02.006>

Liu Y, Mao S, Luo X, Wang Y (2020) Circulating miR-1/UCOA1 is a novel biomarker for the diagnosis and prognosis of acute myocardial infarction. *Int J Cardiol* **310**: 137. <https://doi.org/10.1016/j.ijcard.2020.01.005>

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* **25**: 402-408. <https://doi.org/10.1006/meth.2001.1262>

Peng Y, Zhao JL, Peng ZY, Xu WF, Yu GL (2020) Correction: Exosomal miR-25-3p from mesenchymal stem cells alleviates myocardial infarction by targeting pro-apoptotic proteins and EZH2. *Cell Death Dis* **11**: 845. <https://doi.org/10.1038/s41419-020-03025-4>

Provisiero DP, Negri M, de Angelis C, Di Gennaro G, Patalano R, Simeoli C, Papa F, Ferrigno R, Auremma RS, De Martino MC, Colao A, Pivonello R, Pivonello C (2019) Vitamin D reverts resistance to the mTOR inhibitor everolimus in hepatocellular carcinoma through the activation of a miR-375/oncogenes circuit. *Sci Rep* **9**: 11695. <https://doi.org/10.1038/s41598-019-48081-9>

Qian LL, Liu CQ, Guo YX, Jiang YJ, Ni LM, Xia SW, Liu XH, Zhuang WZ, Xiao ZH, Wang SN, Zhou XY, Sun B, Chinese Collaborative Study Group for Neonatal Respiratory (2010) Current status of neonatal acute respiratory disorders: a one-year prospective survey from a Chinese neonatal network. *Chin Med J (Engl)* **123**: 2769-2775.

Sawires HK, Abdel Ghany EA, Hussein NF, Seif HM (2015) Use of lung ultrasound in detection of complications of respiratory distress syndrome. *Ultrasound Med Biol* **41**: 2319-2325. <https://doi.org/10.1016/j.ultrasmedbio.2015.04.024>

Song H, Lu HN, Chen X, Jiang XF, Yang Y, Feng J (2018) MiR-216a-3p promotes differentiation of BMMSCs into ACE II cells via Wnt/beta-catenin pathway. *Eur Rev Med Pharmacol Sci* **22**: 7849-7857. https://doi.org/10.26355/eurrev_201811_16410

Sweet DG, Carnielli V, Greisen G, Hallman M, Ozek E, Plavka R, Saugstad OD, Simeoni U, Speer CP, Vento M, Halliday HL; European Association of Perinatal Medicine (2013) European consensus guidelines on the management of neonatal respiratory distress syndrome in preterm infants - 2013 update. *Neonatology* **103**: 353-368. <https://doi.org/10.1159/000349928>

Wang S, Li Z, Wang X, Zhang S, Gao P, Shi Z (2021) The role of pulmonary surfactants in the treatment of acute respiratory distress syndrome in COVID-19. *Front Pharmacol* **12**: 698905. <https://doi.org/10.3389/fphar.2021.698905>

Wang Y, Huang C, Reddy Chintagari N, Bhaskaran M, Weng T, Guo Y, Xiao X, Liu L (2013) miR-375 regulates rat alveolar epithelial cell trans-differentiation by inhibiting Wnt/beta-catenin pathway. *Nucleic Acids Res* **41**: 3833-3844. <https://doi.org/10.1093/nar/gks1460>

Wu CP, Bi YJ, Liu DM, Wang LY (2019) Hsa-miR-375 promotes the progression of inflammatory bowel disease by upregulating TLR4.

- Eur Rev Med Pharmacol Sci* **23**: 7543–7549. https://doi.org/10.26355/eurrev_201909_18871
- Xue WX, Zhang MY, Rui Li, Liu X, Yin YH, Qu YQ (2020) Serum miR-1228-3p and miR-181a-5p as noninvasive biomarkers for non-small cell lung cancer diagnosis and prognosis. *Biomed Res Int* **2020**: 9601876. <https://doi.org/10.1155/2020/9601876>
- Yu HR, Li SC, Tseng WN, Tain YL, Chen CC, Sheen JM, Tiao MM, Kuo HC, Huang CC, Hsieh KS, Huang L (2016) Early and late effects of prenatal corticosteroid treatment on the microRNA profiles of lung tissue in rats. *Exp Ther Med* **11**: 753–762. <https://doi.org/10.3892/etm.2016.2992>
- Zhang H, Mishra A, Chintagari NR, Gou D, Liu L (2010) Micro-RNA-375 inhibits lung surfactant secretion by altering cytoskeleton reorganization. *IUBMB Life* **62**: 78–83. <https://doi.org/10.1002/iub.286>
- Zhang YH, Chen AL, Yu RQ, Jia BB, Ye DN, Wang M, Mei YZ, Fang GD, Jiang SY, Zhou Q, Zhang B (2021) miR-296-5p Inhibits the secretion of pulmonary surfactants in pulmonary epithelial cells via the downregulation of Wnt7b/beta-catenin signaling. *Biomed Res Int* **2021**: 4051504. <https://doi.org/10.1155/2021/4051504>
- Zhao L, Shi X, Wang N, Liu C, Wang J (2020) YAP1, targeted by miR-375, enhanced the pro-angiogenesis of airway smooth muscle cells in asthma via STAT3 activation. *Cell Cycle* **19**: 1275–1284. <https://doi.org/10.1080/15384101.2020.1746874>
- Zheng LY, Sun PC (2020) Increased Expression of IL-23 and IL-17 in serum of patients with neonatal respiratory distress syndrome and its clinical significance. *Clin Lab* **66**. <https://doi.org/10.7754/Clin.Lab.2020.191250>
- Zhuo R, Rong P, Wang J, Parvin R, Deng Y (2021) The potential role of bioactive plasmalogens in lung surfactant. *Front Cell Dev Biol* **9**: 618102. <https://doi.org/10.3389/fcell.2021.618102>