European Review for Medical and Pharmacological Sciences 2017; 21: 4167-4180

Roles of hypoxia-inducible factor-1α and its target genes in neonatal hypoxic pulmonary hypertension

C.-F. HAN, Z.-Y. LI, T.-H. LI

Department of Pediatrics, Weifang People's Hospital, Weifang, Shandong Province, China

Abstract. – OBJECTIVE: To investigate the role of hypoxia-inducible factor-1α and its target genes in hypoxic pulmonary hypertension in neonates.

PATIENTS AND METHODS: A total of 117 newborns were selected and divided into two groups for clinical experiments: 85 cases in the hypoxic pulmonary hypertension (HPH) group, including mild, moderate and severe subgroups, and 32 cases in the case-control group. ELISA was used to detect the serum HIF-1α, endothelin-1 (ET-1) and adrenomedullin (ADM) levels, and echocardiography was used to detect the dynamic changes in pulmonary artery systolic pressure (PASP), right ventricular ejection fraction (RVEF), tricuspid E peak and A peak ratio (E/A) and right ventricular Tei index.

RESULTS: The average PASP level of the HPH group was significantly higher than that of the control group at 1 d and 3 d after birth ($p < 0.05$). The average PASP level was still higher in the severe HPH group than that in the control group at 7 d after birth, while the average levels in the mild and moderate HPH groups recovered to the normal. Compared with those in control group, RVEF and E/A of the tricuspid valve were decreased significantly in severe HPH patients ($p < 0.05$). The Tei indexes of the right ventricle were significantly higher in the mild, moderate and severe HPH groups than those in control group and the right ventricular Tei index was positively correlated with PASP. The levels of serum ADM, HHH-1α and ET-1 in all the three HPH subgroups were significantly higher than those in the control group at 1 d after birth and showed positive correlations with PASP ($p < 0.05$), except that serum ADM in mild HPH showed no obvious difference from the control group. The levels of serum HIF-1α and ADM in the severe HPH group and the ET-1 levels in the moderate and severe groups were increased significantly at 3 d after birth ($p < 0.05$).

CONCLUSIONS: The PASP level in neonates with HPH is related to the serum HIF-1α, ET-1 and ADM levels, indicating that hypoxia can increase the level of HIF-1α, which in turn will enhance the expression of downstream target genes ET-1 and ADM, further leading to pulmonary hypertension. The right ventricular Tei index can be used to sensitively detect right ventricular dysfunction of mild, moderate and severe HPH groups.

Key Words:
Hypoxia-inducible factor, Endothelin-1, Adrenomedullin, Hypertension, Neonate.

List of Abbreviations

HIF-1α: Hypoxia-inducible Factor-1α; HPH: hypoxic pulmonary hypertension; ET-1: endothelin-1; ADM: adrenomedullin; PASP: pulmonary artery systolic pressure; RVEF: right ventricular ejection fraction; E/A: tricuspid E peak and A peak ratio; RDS: respiratory distress syndrome.

Introduction

Hypoxic diseases, such as neonatal asphyxia, infectious pneumonia, and respiratory distress syndrome (RDS), are common diseases in neonates[1]. A variety of hypoxic diseases lead to a decrease in the alveolar oxygen content in pulmonary arteries and alveoli before and after birth. With the involvement of a series of vasoactive substances and humoral factors, arterial vasoconstriction in the lungs increases, resulting in increased pulmonary vascular resistance, and eventually leading to increased pulmonary artery pressure, which is clinically known as neonatal hypoxic pulmonary hypertension (HPH)[2,3]. When severe persistent hypoxia occurs, the right ventricular pressure increases so that the newborn's pulmonary circulation pressure exceeds the systemic arterial pressure, with the consequential

Corresponding Author: Tianhua Li, MD; e-mail: hk46he@163.com

persistence of the circulation through the *ductus arteriosus* or the *foramen ovale* into the systemic circulation bypassing the lungs and leading to serious clinical hypoxemia. During the whole process, the release of vasoactive factors due to vascular injury can mediate persistent pulmonary vasoconstriction, spasm and the occurrence of remodeling. This stage is known as persistent pulmonary hypertension of the newborn (PPHN)[4]. Studies are still needed to investigate the pathogenesis of neonatal HPH. A research[4] in adults suggests that hypoxia can lead to pulmonary microvascular endothelial injury, imbalance of vasomotor factors from endothelial secretion, pulmonary vasoconstriction (HPV) and pulmonary hypertension. In the process of HPH, hypoxia-inducible factor-1α (HIF-1α) can regulate expression of the contraction factor endothelin-1 (ET-1) and diastolic factor adrenomedullin (ADM), leading to an imbalance between ET-1 and ADM, which has been implicated in the mechanism of pulmonary hypertension[5]. Current clinical treatments of HPH have different targets, focusing on treatment of any clear causal factors and prevention of complications. However, the recurrence rates are high[5]. Understanding the role of HIF-1α and its downstream genes in the development of neonatal HPH may help produce more new successful treatment approaches. Cardiac catheterization, which can be used to measure pulmonary artery pressure directly, has important diagnostic value for HPH, but is not suitable for neonates. In contrast, Doppler echocardiography, a noninvasive technique, can reveal the right-to-left shunt at the level of the oval foramen and/or the arterial catheters. Also, it can quantify pulmonary artery pressure, and it has become the most important newborn HPH diagnostic tool, and is also widely used in the evaluation of HPH treatment[5]. A prominent clinical feature of neonatal HPH is that it can easily incorporate right ventricular dysfunction, which is usually difficult to be detected at the early stages if not suspected, but it will dramatically decrease the efficiency of the treatment and results in a poor prognosis once established. Conveniently, echocardiography is well suited to diagnose the early stages of cardiac dysfunction[5]. Nevertheless, the routine echocardiographic examination in newborns (right ventricular ejection fraction RVEF, tricuspid E peak and A peak ratio E/A) has limitations, and the right ventricular Tei index in the new echocardiographic approach used in adults is considered as a promising indicator of overall right ventricular function[5]. Studies on the right ventricle of neonates with HPH have revealed that the right ventricular Tei index has promising application value for the early detection of right ventricular dysfunction in neonatal HPH, and is also clinically valuable for evaluating the early treatment and prognosis of neonatal HPH[6]. In order to help elucidate the pathogenesis of neonatal hypoxic pulmonary hypertension, we measured the expressions of serum HIF-1α, ET-1 and ADM in neonates with HPH and healthy newborns used as controls. We also used echocardiographic routine methods to detect the dynamic changes of pulmonary artery systolic pressure (PASP), right ventricular ejection fraction (RVEF) and tricuspid E/A ratio. The right ventricle Tei indexes were also dynamically monitored.

Patients and Methods

Patients

During the period from June 2013 to May 2015, 117 full-term newborn infants were enrolled in the study after admission to Weifang People's Hospital. The patients were divided into the case and control group. Out of all the 117 cases, acute respiratory distress syndrome was detected in 40 cases, pneumonia was found in 18 cases, severe ventricular septum in 12 cases, fetal aspiration syndrome in 11 cases and sepsis in 4 cases. The research team obtained the approval of the Ethics Committee of our hospital.

Case Group (HPH Group)

This group included a total of 85 neonates with HPH admitted to the Department of Neonatology of Weifang People's Hospital (Weifang, Shandong Province, China). There were 41 males and 44 females. The gestational age was ranged from 37-42 weeks with a mean value of 38.1 ± 2.5 weeks. The birth weight was ranged from 2550 to 3980 g with a mean value of 3105 ± 312 g. All the patients were treated as appropriately for their primary disease, with additional oxygen breathing support and symptomatic treatment as needed.

Inclusion Criteria of Case Group

(1) Patients with a history of clear perinatal hypoxia and hypoxia symptoms: cyanosis of skin or lips, abnormal respiratory rate or rhythm, and with labored breathing with the three concave syndromes, etc.; patients with neonatal hypox-

emia (PaO_2 < 50 mmHg) via blood gas analysis and the pulse oxygen saturation ($TcSaO_2$) of lower than 85%. (2) Patients who had been hospitalized for more than 7 days with active clinical treatment, whose serum HIF-1α, ET-1 and ADM samples were taken at 1, 3 and 7 d after birth; whose pulmonary artery systolic pressure was checked by Doppler echocardiography, RVEF, tricuspid E/A ratio was determined by conventional ultrasound technology, and right ventricular Tei indexes were available. (3) Patients receiving Doppler echocardiography, whose pulmonary artery systolic pressure was estimated by tricuspid regurgitation, conduit pressure difference and pulmonary hypertension were determined by ultrasound technology and the PASP value was higher than 40 mmHg. Based on the degree of the increase in PASP value, the patients in the HPH group were further divided into three subgroups: 38 cases in mild HPH (40 mmHg < PASP < 50 mmHg), 27 cases in moderate HPH (50 mmHg < PASP < 70 mmHg) and 20 cases in severe HPH (PASP ≥ 70 mmHg).

Exclusion Criteria of Case Group

Newborns with neonatal bronchial and pulmonary vascular dysplasia, congenital heart disease and/or congenital malformations were excluded. Patients with pulmonary hypertension, those who died within 7 days after birth and/or those with missed blood samples and/or missed Doppler echocardiographic data, were also excluded.

Control Group

The full-term newborn infants after admission to Weifang People's Hospital were selected during the same period as the case group. All the newborns in the control group had clear hypoxia phenotypes or cardiopulmonary diseases. The birth weight and gestational age were close to those of HPH group. For the 32 cases selected, there were 17 males and 15 females. The gestational age was ranged from 37-42 weeks with a mean value of 38.2 ± 2.2 weeks. The birth weight was ranged from 2500-4000 g with a mean value of 2980 ± 314 g. 2). The patients had been hospitalized for more than 7 days with active clinical treatment. The serum HIF-1α, ET-1 and ADM samples were taken at 1, 3 and 7 d after birth. Also, complete information of pulmonary artery systolic pressure was checked by Doppler echocardiography, RVEF, tricuspid E/A ratio was determined by conventional ultrasound technology, and right ventricular Tei indexes were available. No pulmonary arterial hypertension was detected by Doppler echocardiography (PASP < 40 mmHg).

Exclusion Criteria of Control Group

The exclusion criteria were the same as those of the case group.

Major Instruments and Reagents

Doppler ultrasound diagnostic apparatus (color digital): GEVI 1D 7 type (GE, Boston, MA, USA); pediatric ultrasound probe (4 MHZ-8 MHZ frequency phased array probe); 2 mm microplate reader for Doppler ultrasound diagnosis: 550 microplate reader (BioRad Model, Osaka, Japan); intelligent electric constant temperature incubator DHP-9160B (Shanghai Boxuan Laboratory Equipment, Shanghai, China); Oscillator (MM-2 type) (Jintan Medical Instrument Factory, Tianjin, China); Centrifuge (SC-2546 type) (Zhongjia Constituent Company of Anhui USTC Chuangxin, Anhui, China); refrigerator (-4°C) (Haier, Qingdao, China); refrigerator (-86°C) Thermo Fisher Scientific (Waltham, MA, USA); micropipette, Thermo Fisher Scientific (Waltham, MA, USA).

The Main Reagents for ELISA Tests

Freeze-dried standards (Zhongshan Golden-Bridge, Beijing, China): 2 bottles, each bottle was diluted into 1 mL using sample diluent. A series fold dilution was prepared to make a series of dilutions including 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL and 15.6 pg/mL. The stock solution was used as the highest standard concentration. Sample dilution was used as the lowest standard concentration of 0 pg/mL. All dilutions were prepared within 15 min before use.

Ultrasonic Testing Methods

PASP Detection Via Echocardiography

To avoid moving and disturbing the patients, the portable Doppler ultrasonic apparatus was used for bedside echocardiography at 1, 3 and 7 d after birth. As the subjects were required to keep quiet, formula milk was used to feed and comfort crying infants. Sedatives were not used to calm down the patients. The procedure was only performed after the patients became quiet. Under the supine position, a standard section exploration was done on the sternum, xiphoid,

apex and clavicles with video recording done at the same time. All images were stored in Real-time on the magneto-optical disk for image playback measurement and analyses. Doppler echocardiography was applied using a continuous wave Doppler technique to record the tricuspid regurgitation spectrum, and measure the maximum reflux velocity. The right ventricular outflow tract obstruction was combined and/or at the condition of pulmonary artery stenosis (no pressure difference between the right ventricular and pulmonary artery, right ventricular systolic pressure was equal to pulmonary artery systolic pressure). TRPG was used to determine pulmonary artery systolic pressure. The measurement methods were as follows: The peak velocity (Vm) of tricuspid regurgitation was determined, and PASP was calculated according to the simplified Bernoulli equation: PASP = TRPG + 10 mmHg. When the tricuspid regurgitation was not measured, continuous wave Doppler technique was used to record the shunt spectrum at the *foramen ovale* or arterial catheters. That is, according to the maximum speed of the bypass flow, the maximum pressure difference (AP) between the systole of pulmonary artery and aorta was calculated, and the corresponding calculation of PASP was also done according to the formula: PASP = systolic blood pressure of the aorta -ΔP, in which aortic systolic blood pressure could be replaced by brachial artery pressure.

Conventional Ultrasonic Testing

Measurement of RVEF

Right ventricular end-diastolic and right ventricular end-systolic volumes were measured by two-dimensional echocardiography with the patients in bed according to the Simpson method of double plane. The RVEF value was calculated to evaluate the systolic function of the right ventricle: RVEF = (right ventricular end-diastolic volume-right ventricular end-systolic volume)/right ventricular end-diastolic volume × 100%.

Measurement of Tricuspid Flow Spectrum E and A Peaks and Calculation of E/A Ratio

The standard, apical four-chamber section view was selected for measurements. The pulsed Doppler ultrasonic sampling probe was placed in the tricuspid valve mouth to detect the tricuspid valve blood flow spectrum: the E peak of the early right ventricular diastolic tricuspid valve and the A peak of the late right ventricular diastolic tricuspid valve were measured. The E/A ratio was calculated to evaluate the diastolic function of the right ventricle.

Measurement of Right Ventricular Tei Index (RV-Tei)

The pulse Doppler ultrasound-sampling probe was placed in the tricuspid valve tip in the direction of apical four-chamber section to record the tricuspid valve flow spectrum. The pulsed Doppler sampling was placed under the pulmonary valve in the direction of parasternal short axis section to record the blood flow spectrum of the right ventricular outflow tract.

Measurement of Serum HIF-1α, ET-1, and ADM Levels

The pulmonary artery systolic pressure of the neonates in both the case and control groups was detected by portable Doppler echocardiography at 1, 3 and 7 d after birth. At the same time, 2 mL blood samples were collected from the peripheral vein and left standing for 30 min until solid. Each sample was then centrifuged at 3000 rpm for 10 min at room temperature. The supernatants were taken, sealed and placed in a -4°C refrigerator for storage. The levels of serum HIF-1α, ET-1 and ADM were measured by ELISA. Before measurement, the frozen samples were thawed at room temperature. The samples were well shaken before measurements and multiple freeze-thaw cycles were avoided. The ELISA kit was provided by Shanghai Westang Bio-Tech Inc., (Shanghai, China).

Quality Control

Cardiac ultrasonography was performed for patients beside the bed. The same physician with extensive echocardiographic experience performed all cardiac echocardiographic measurements. Three cardiac cycles were measured consecutively, and the resulting values were averaged. The physician made sure that the patients kept quiet during the procedure to avoid heart rate fluctuations resulting from inadequate movements. Formula milk was offered to feed and comfort the crying patients. The ultrasound beam was parallel to the apical four chamber section when the Doppler detection was being performed; if the angle between the blood stream and the sample line was greater than 30°, the angle should be corrected as much as possible

to make them parallel to each other. In order to reduce errors, all the saved video were replayed by the examining physician once again to further confirm the ultrasound data after cardiac ultrasonography was performed. The single-blind principle was used for the whole procedure of cardiac ultrasonography, that is, cardiac ultrasound physicians completed the testing work without being informed of the group the subjects belonged to. Hemolysis was avoided in neonatal serum specimens. All the required reagents were prepared before the measurement of serum HIF-1α, ET-1 and ADM levels. During reagent preparation and sample dilution, the mixtures were mixed well. Fresh standard solutions were used for each test, and the bottom plate should be kept clean to ensure the accuracy and reliability of results. A standard curve was drawn for each test. If the concentration was too high, the sample should be diluted with the sample dilution, so that the concentration of the samples was kept in line with the detection range required by the kit. All testing operations were only carried out by specified professional individuals in strict accordance with the operational requirements or instruction procedures. The experimental time was controlled to avoid effects from extended reaction times. The single-blind principle was used for the whole procedure of cardiac ultrasonography, that is, cardiac ultrasound physicians completed the testing work without being informed of the group the subjects belonged to.

Statistical Analysis

Statistical analysis was performed with SPSS 18.0 (IBM, Armonk, NY, USA) statistical software packages. Measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis of the general data, the comparison of the levels of serum factors, pulmonary artery systolic pressure, RVEF, and tricuspid valve E/A ratio and right ventricular Tei index among groups, were performed using the analysis of variance with multiple sample averages. The variance homogeneity test was carried out when multiple groups were compared. The SNK-q test (or the LSD-*t* test) of the two groups was used to compare levels of serum HIF-1α, ET-1, ADM and pulmonary artery systolic pressure between every two groups in the three HPH groups and control group at the same time point. Linear correlation analysis and partial correlation analysis between two variables were used to analyze the correlation of the levels of HIF-1α, ET-1, ADM, and right ventricle Tei index in each group with pulmonary artery systolic pressure. $p < 0.05$ was considered to be statistically significant.

Results

Clinical Data of Study Subjects

Variance analysis showed no significant differences in gestational age and birth weight between the case and control groups ($F_{\text{gestational age}} = 1.13$, $p > 0.05$; $F_{\text{body weight}} = 0.67$, $p > 0.05$). The general situation of each group could be considered to be comparable. The results are shown in Table I.

Comparison of PASP Value Between the Case and Control Groups

Variance homogeneity tests showed the variance of each group being similar. Using the analysis of variance of multiple sample average comparison, $p < 0.05$ suggested that the difference of PASP value between the control group and each case group could be considered to be statistically significant. If the population mean was not equal or not totally equal, SNK-q test was used for multiple comparisons. The results showed that the PASP value of case group was statistically different from that of the control group ($p < 0.01$, $p < 0.05$). There were significant differences in the PASP value at 1 d and 3 d after birth ($p < 0.01$). Only the PASP value of the severe HPH group showed significant difference

Table I. Comparison of gestational age and body weight of neonates between case and control groups ($\bar{x} \pm s$).

Group	No.	Body weight (g)	Gestational age (w)
Control	32	2980 ± 314	38.2 ± 2.2
Mild HPH group	38	2987 ± 218	38.9 ± 2.6
Moderate HPH group	27	3216 ± 264	39.3 ± 2.8
Severe HPH group	20	3073 ± 256	38.5 ± 1.9
F or (χ^2)	–	1.13	0.67
p value	–	0.56	0.96

Table II. Comparison of PASP value between case and control groups ($\bar{x} \pm s$).

Groups	No.	PASP (mmHg)		
		1 day after birth	3 days after birth	7 days after birth
Control	32	24.64 ± 8.78	25.42 ± 11.15	22.33 ± 6.01
Mild HPH group	38	46.97 ± 7.21[a]	45.55 ± 7.03[a]	23.45 ± 4.28
Moderate HPH group	27	60.58 ± 7.92[a,d]	64.18 ± 9.15[a,d]	27.68 ± 7.46
Severe HPH group	20	81.07 ± 12.1[a,d,e]	81.49 ± 7.65[a,b,c]	43.36 ± 9.20[c,d,e]
F or (χ^2)	–	34.28	37.25	4.43
p-value	–	< 0.01	< 0.05	< 0.05

[a]Comparison with control group, $p < 0.01$; [b]Comparison with the mild group, $p < 0.01$; [c]Comparison with the control group, $p < 0.05$; [d]Comparison with mild group $p < 0.05$; [e]Comparison with the moderate group, $p < 0.05$.

from that of the control group at 7 d after birth ($q = 4.22$, $p < 0.05$). Significant differences in PASP value were found between every two groups in the three HPH groups at 1 d and 3 d after birth ($p < 0.05$); but significant differences in PASP value were only found between severe and mild groups and severe and moderate groups at 7 d after birth ($p < 0.05$). The PASP value of the mild group showed no significant difference from that of the moderate group ($q = 1.04$, $p > 0.05$) (Table II).

Comparisons of Right Ventricular Function Indexes (RVEF, Tricuspid Valve E/A ratio) Between the Case and Control Groups Using Neonatal Routine Ultrasound

The RVEF value and tricuspid valve E/A ratio of the case and control groups were detected by neonatal routine ultrasound. Variance homogeneity test showed that the variance of each group was similar, so analysis of variance was used for comparison. If the *p*-value of an overall mean in one of the four groups was lower than 0.05, they were considered not all equal, thus the mean value of RVEF and tricuspid valve E/A ratio of each HPH subgroup in each case group and control group received the LSD-*t* test (Table III). The RVEF value and tricuspid valve E/A ratios in the above-mentioned Tables and Figures of the mild and moderate HPH group showed no significant differences from those of the control group at 1 d, 3 d and 7 d after birth ($p > 0.05$). However, the RVEF value and tricuspid valve E/A ratios of the severe HPH group were significantly lower than those of the control group ($p < 0.01$, $p < 0.05$), indicating that the right ventricular systolic and diastolic functions of the patients in the severe HPH group are weaker than those of the control group at 1 d, 3 d and 7 d after birth.

Comparison of RV-Tei Between Case and Control Groups

The RV-Tei of neonates in the case and control groups was analyzed by variance test. The homogeneity test of variance showed that the variance of each group was similar to each other. $p < 0.05$ suggested that the overall means of the four groups were not totally equal. Thus, the RV-Tei of the case group was analyzed and compared with that of the

Table III. Comparison of RVEF value and tricuspid valve E/A ratio between case and control groups ($\bar{x} \pm s$).

Groups	No.	RVEF (%)			E/A		
		1 day after birth	3 days after birth	7 days after birth	1 day after birth	3 days after birth	7 days after birth
Control group	32	45.58 ± 10.67	48.25 ± 12.94	49.37 ± 9.83	1.05 ± 0.23	1.02 ± 0.19	1.21 ± 0.14
Mild HPH group	38	43.79 ± 12.13	46.67 ± 11.66	47.39 ± 10.17	0.94 ± 0.31	0.98 ± 0.23	1.05 ± 0.19
Moderate HPH group	27	40.19 ± 11.16	41.45 ± 9.59	44.72 ± 8.19	0.96 ± 0.29	0.91 ± 0.21	0.99 ± 0.20
Severe HPH group	20	34.19 ± 10.37[a]	35.24 ± 11.0[a]	40.17 ± 12.49	0.81 ± 0.21[a]	0.76 ± 0.16[a]	0.89 ± 0.24[b]
F		6.79	5.16	3.75	9.75	8.01	5.42
p-value		< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05

[a]Comparison with control group, $p < 0.01$; [b]Comparison with the mild group, $p < 0.01$; [c]Comparison with the control group, $p < 0.05$; [d]Comparison with mild group $p < 0.05$; [e]Comparison with the moderate group, $p < 0.05$.

control group using LSD-t test (Table IV). The RV-Tei values of the case group were higher than that of the control group at 1 d and 3 d after birth ($p < 0.01$). The RV-Tei values of the mild and moderate groups showed no significant differences from that of the control group at 7 d after birth (t = 2.17/2.35, $p > 0.05$), but the RV-Tei value of the severe group was still higher than that of the control group at the same time point (t = 4.86, $p < 0.05$) (Table IV).

Analysis of the Correlations Between RV-Tei Index and PASP Value in the Case and Control Groups

The RV-Tei index and PASP value of the case and control groups were analyzed by linear correlation. As can be seen from Table V, the RV-Tei index of the case group was positively correlated with PASP (r = 0.69, r = 0.71, $p < 0.05$) at 1 d and 3 d after birth, but showed no correlation at 7 d after birth (r = 0.32, $p > 0.05$). The RV-Tei index of the control group showed no correlation with PASP at 1 d, 3 d and 7 d after birth ($p > 0.05$) (Table V).

Comparisons of Serum HEF-1α, ET-1 and ADM Levels Between the Case and Control Groups

The levels of serum HIF-1α, ET-1 and ADM were compared between the case and control groups. The serum levels of HIF-1α, ET-1 and ADM between different time points in each group were compared by the analysis of variance of repeated measurement data. Homogeneity test of variance showed that the variance of each group was similar, so the multiple sample average comparison was applied. It was considered that the population means of the case and control groups were not equal or not totally equal since not all p-values were higher than 0.05. The SNK-q test was used for multiple comparisons between the control group and the subgroups of case groups. The results are shown in Tables VI, VII and VIII, respectively. As can be seen from Table VI, the serum HIF-1α level of the case group was higher than that in the control group at 1 d after birth (F = 31.76, $p < 0.01$). In the three HPH subgroups, the HIF-1α level of the severe group was significantly higher than that of the moderate group (Q = 4.74, $p < 0.05$) and the HIF-1α level of the moderate group was significantly higher than that of the mild group ($q = 4.35$, $p < 0.05$). The serum HIF-1α level of the mild and moderate groups showed no significant difference from that of the control group at 3 d after birth ($p > 0.05$), but there was a significant difference between the severe HPH group and the control group ($q = 4.07$, $p < 0.05$). The serum HIF-1α level of all the three HPH groups showed no significant difference from that

Table IV. Comparison of RV-Tei value between case and control groups ($\bar{x} \pm s$).

Groups	No.	RV-Tei		
		1 day after birth	3 days after birth	7 days after birth
Control group	32	0.40 ± 0.07	0.38 ± 0.09	0.38 ± 0.12
Mild HPH group	38	0.56 ± 0.06[a]	0.53 ± 0.07[a]	0.42 ± 0.08
Moderate HPH group	27	0.65 ± 0.07[a]	0.64 ± 0.14[a]	0.45 ± 0.10
Severe HPH group	20	0.78 ± 0.10[a]	0.79 ± 0.12[a]	0.61 ± 0.15[b]
F		48.23	63.08	32.11
p value		< 0.01	< 0.01	< 0.05

[a]Comparison with control group, $p < 0.01$; [b]Comparison with control group, $p < 0.5$

Table V. Correlation analysis between RV-Tei index and PASP value in the case and control groups.

	PASP					
	Case group (n = 85)			Control group (n = 32)		
Index	1 day after birth	3 days after birth	7 days after birth	1 day after birth	3 days after birth	7 days after birth
RV-Tei Index	0.69	0.71	0.32	0.21	0.24	0.2
p-value	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	< 0.05

Table VI. The comparison of serum HEF-1α level between case and control groups ($\bar{x} \pm s$, pg/ml).

Group	No.	1 day after birth	3 days after birth	7 days after birth
Control group	32	282.74 ± 40.17	241.27 ± 31.46	224.34 ± 37.38
Mild HPH group	38	597.43 ± 75.37[a]	275.78 ± 10.26	190.27 ± 41.36
Moderate HPH group	27	856.89 ± 107.41[a,d]	280.19 ± 61.65	242.36 ± 80.21
Severe HPH group	20	1039.84 ± 212.26[a,c,e]	855.36 ± 94.58[a,d,e]	278.45 ± 79.15
F	–	31.76	21.37	5.78
p-value	–	< 0.01	< 0.01	> 0.05

[a]Comparison with control group, $p < 0.01$; [b]Comparison with control group, $p < 0.05$; [c]Comparison with the mild group, $p < 0.01$; [d]Comparison with mild group, $p < 0.05$; [e]Comparison with the moderate group, $p < 0.05$.

Table VII. The comparison of serum ET-1 level between case and control groups ($\bar{x} \pm s$, pg/ml).

Group	No.	1 day after birth	3 days after birth	7 days after birth
Control group	32	44.24 ± 7.51	40.17 ± 11.43	31.49 ± 14.59
Mild HPH group	38	89.38 ± 18.74[b]	50.69 ± 13.17	45.73 ± 21.64
Moderate HPH group	27	207.60 ± 32.97[a,d]	179.45 ± 27.51[a,d]	57.70 ± 17.37
Severe HPH group	20	370.34 ± 41.15[a,c]	855.36 ± 94.58[a,d,e]	278.45 ± 79.15
F	–	28.45	24.43	6.73
p-value	–	< 0.01	< 0.01	< 0.05

[a]Represents the comparison with control group, $p < 0.01$; [b]Represents the comparison with control group, $p < 0.05$; [c]Comparison with the mild group, $p < 0.01$; [d]Comparison with mild group, $p < 0.05$; [e]Comparison with the moderate group, $p < 0.05$.

of the control group at 7 d after birth (F = 5 .78, $p > 0.05$). As shown in Table VII, the serum ET-1 level of the case group was higher than that in the control group at 1 d after birth (F = 28.45, $p < 0.01$). In the three HPH subgroups, the ET-1 level of the severe group was significantly higher than that of the moderate group ($q = 4.36$, $p < 0.05$) and the ET-1 level of the moderate group was significantly higher than that of the mild group ($q = 4.17$, $p < 0.05$). The serum ET-1 level of the mild group showed no significant difference from that of the control group at 3 d after birth ($p > 0.05$), but the ET-1 level of moderate and severe HPH groups showed significant difference from that of the control group ($p < 0.05$). The serum ET-1 level of the severe group was higher than that of the moderate group at 3 d after birth ($q = 5.14$, $p < 0.05$) and the level of the moderate group was higher than that of the mild group at the same time point ($q = 5.35$, $p < 0.05$). The serum ET-1 level of the severe group was still higher than that of the control group at 7 d after birth ($q = 4.04$, $p < 0.05$), but the level of the moderate and mild groups showed no significant difference from that of the control group ($p > 0.05$). As can be seen in Table VIII, significant differences of serum ADM levels were found between the case and control groups at 1 d and 3 d after birth (F = 35.02/25.65, $p < 0.05$). The serum ADM level in the severe group was higher than that in the control group ($p < 0.01$). The ADM level in the severe group was also higher than that in the moderate group (q =

Table VIII. The comparison of serum ADM level between case and control groups ($\bar{x} \pm s$, U/L).

Group	No.	1 day after birth	3 days after birth	7 days after birth
Control group	32	20.47 ± 13.07	16.46 ± 9.86	18.48 ± 5.59
Mild HPH group	38	25.64 ± 11.65	23.34 ± 12.44	22.45 ± 8.18
Moderate HPH group	27	42.34 ± 17.64[b]	27.37 ± 14.58	20.47 ± 9.57
Severe HPH group	20	67.72 ± 12.47[a]	57.64 ± 10.69[a]	21.64 ± 8.52
F	–	35.02	25.65	3.35
p-value	–	< 0.01	< 0.05	> 0.05

[a]Comparison with control group, $p < 0.01$; [b]Comparison with control group, $p < 0.05$.

4.09, $p < 0.05$). The level of the case group was significantly higher than that in control group at 3 d after birth (q = 4.16, $p < 0.01$), but no statistically significant difference was found between every two groups of other groups at the same time point ($p > 0.05$).

Correlation Analysis Between the PASP value and the Levels of Serum HIF-la, ET-1 and ADM in the Case and Control Groups

Linear correlation and partial correlation analyses between two variables were applied to analyze the correlations between the PASP value and the levels of serum HIF-1α, ET-1 and ADM in the case and control groups. As can be seen from Table IX, a positive correlation was found between PASP and the serum HIF-1α, ET-1 and ADM levels in the case group at 1 d after birth. The correlation coefficient value between PASP and HIF-1α was the highest (r = 0.75, $p < 0.01$). A positive correlation was found between PASP and the serum ET-1 level in the case group at 3 d after birth (r = 0.61, $p < 0.01$), but no correlation was found between PASP and the serum HIF-1α/ADM level at the same time point. There was no correlation between PASP and the levels of serum HIF-1α, ET-1 and ADM at 7 d after birth ($p > 0.05$). Because of a possible correlation between the serum HIF-1α, ET-1, and ADM levels, a partial correlation analysis between PASP and levels of serum HIF-1α, ET-1, and ADM, was performed. The results in Table X are consistent with those of the linear correlation analysis. There were positive correlations between PASP and the levels of serum HIF-1α, ET-1 and ADM at 1 d after birth. Furthermore, the partial correlation coefficient between PASP and HIF-1α was the highest (r = 0.77, $p < 0.01$). At 3 d after birth, only PASP was positively correlated with ET-1 level (r = 0.65, $p < 0.01$). There was no correlation between PASP and the levels of serum HIF-1α, ADM and ET-1 in other groups ($p > 0.05$). No correlation was found between PASP and levels of serum HIF-1α, ADM and ET-1 in the control group after linear correlation and partial correlation analysis ($p > 0.05$).

Discussion

Because of its complex etiology, difficult diagnosis and treatment, and the poor prognosis, pulmonary artery hypertension (PAH) has become a hot worldwide topic for research. During the development of hypoxia in neonates, hypoxic pulmonary vasoconstriction and vascular resistance (HPV and HPVR) are two important pathological processes. Hypoxia leading to the occurrence of HPV and HPVR in newborns is called neonatal HPH. Pulmonary artery pressure increases persistently if hypoxia is not corrected in time. Once the pulmonary circulation pressure exceeds the systemic arterial circulation pressure, the fetal cycle will continue the right-left blood shunt through the atrial *foramen ovale* and/or the *ductus arteriosus*. The systemic blood oxygen content decreases and clinical severe hypoxemia arises. This phenotype can be accompanied by right heart or whole heart failure. When it advances to persistent pulmonary hypertension of the newborn, the high mortality rate will be inevitable. Timely diagnosis and treatment are necessary[7]. In order to develop more efficacious treatment options against HPH, it is necessary to elucidate the pathogenesis of the disease. The first

Table IX. Linear correlation analysis between PASP value and levels of serum HIF-la, ET-1 and ADM in case and control groups.

	PASP					
	Case group (n = 85)			Control group (n = 22)		
Index	1 day after birth	3 days after birth	7 days after birth	1 day after birth	3 days after birth	7 days after birth
HIF-1α	0.75 < 0.01	0.38 > 0.05	0.3 > 0.05	0.15 > 0.05	0.31 > 0.05	0.24 > 0.05
E-1	0.56 < 0.05	0.61 < 0.05	0.31 > 0.05	0.21 > 0.05	0.23 > 0.05	0.3 > 0.05
ADM	0.34 < 0.01	0.12 > 0.05	0.17 > 0.05	0.3 > 0.05	0.14 > 0.05	0.12 > 0.05

Table X. Linear correlation analysis between PASP value and levels of serum HIF-la, ET-1 and ADM in case and control groups.

	PASP					
	Case group (n = 85)			Control group (n = 22)		
Index	1 day after birth	3 days after birth	7 days after birth	1 day after birth	3 days after birth	7 days after birth
HIF-1α	0.77 < 0.01	0.41 > 0.05	0.35 > 0.05	0.11 > 0.05	0.14 > 0.05	0.2 > 0.05
E-1	0.59 < 0.05	0.65 < 0.05	0.34 > 0.05	0.21 > 0.05	0.2 > 0.05	0.17 > 0.05
ADM	0.31 < 0.01	0.10 > 0.05	0.16 > 0.05	0.04 > 0.05	0.09 > 0.05	0.12 > 0.05

important step is to focus on the cytokine changes and the roles of hypoxia-inducible factor HIF-1α and its downstream genes, which all seem to play important roles in the pathogenesis of HPH. Pulmonary hypertension can increase the right ventricular load, induce the ventricular dilatation, decrease the coronary blood perfusion, develop the hypoxia and ischemia of subendocardial myocardium and finally papillary muscle will be affected, resulting in eventual myocardial cell damage[8]. The function of the right ventricle affects the function of the entire circulatory system[9,10]. Severe right ventricular dysfunction will lead to whole heart failure and high mortality. Therefore, research has focused on functional studies of the right ventricle. The ejection fraction (EF) is still considered as an important indicator of ventricular systolic function. The left ventricular EF lower than 50% indicates left ventricular systolic dysfunction; but there are no clear boundaries for abnormal right heart EF and further studies are needed. Ultrasonic tests of the right heart are complicated due to the right ventricle's irregular structure and its location in the back of thoracic sternum[11]. Due to the independent narrow right ventricular outflow tract, it is difficult to get a satisfactory geometric shape, something easier to do for the left ventricle. In addition, the right ventricular endocardial surface is not regular, making it difficult to make ultrasonic measurements[12]. So the RVEF does not accurately reflect the systolic function of the right ventricle. In addition, clinical examination is affected by body position of the subjects, which leads to inconsistent data and low reproducibility when examining infants[13]. In this study, in addition to the RVEF, the diastolic E peak, A peak and E/A ratio were also used to reflect the filling conditions of right ventricle diastolic pressure and right ventricular compliance. The tricuspid valve flow spectrum can be used to detect the E peak caused by rapid filling in the early stage of the ventricular diastole, and the A peak caused by the right ventricular systole and filling. Therefore, the E/A ratio represents a change in the right ventricular filling rate, which in turn is a better indicator of cardiac diastolic function in adults and older children. The normal right ventricular function of healthy adults[14] makes the E/A ratio higher than 1. However, the E peak of PAH patients is low due to early diastolic rapid filling damage, leading to the increased late diastolic filling for compensation and increased A value. The E/A ratio will decrease, suggesting a combination of PAH and right ventricular diastolic dysfunction[15]. The right ventricular diastolic function of early newborns is not fully developed and the E/A ratio is variable. The E/A ratio becomes increasingly more stable with the development of the myocardial tissue[16]. The Tei index is widely used in adult clinical research and is considered as a valuable indicator of whole ventricular function. Both Right ventricular diastolic function suppression and systolic function inhibition can lead to changes in the right ventricular Tei index[17]. The Tei index increase can be caused by cardiac diastolic dysfunction, IRT extension, systolic dysfunction, ICT extension or ET shortening[17]. Therefore, the Tei index should be considered as a promising index in the comprehensive evaluation of cardiac systole and diastole. Hypoxia-induced pulmonary microvascular endothelial injury is the first step in pulmonary hypertension[18]. Vascular endothelial cells play pivotal roles in the maintenance of normal blood vessel development and growth balance. As the body's largest endocrine organ, the endothelium

can synthesize and secrete a variety of cytokines and growth factors to maintain internal balance. Pulmonary vasospasm and contraction are the two main manifestations of early hypoxic pulmonary hypertension in neonates[18]. The balance between the vasoconstrictor endothelin-1 (ET-1) and the diastolic factor adrenomedullin (ADM) plays an important role in the occurrence and development of pulmonary hypertension in neonates[19]. Hypoxic pulmonary hypertension experiments in animal models have shown that HIF-1α can regulate the expression of ET-1 and ADM, thus participating in the formation of pulmonary hypertension[20]. Compared with wild-type mice, the formation of pulmonary hypertension, pulmonary vascular structural remodeling, right ventricular hypertrophy and other lesions of heterozygous HIF-1α deficient mice are significantly blocked after 6 weeks in a hypoxic environment[21]. Echocardiography can be used to observe the changes of pulmonary arterial pressure and related diagnostic indexes during neonatal hypoxia from a macro perspective. Combined with the observation of the changes in HIF-1α, ET-1, ADM and other inflammatory factors, it provides valuable clinical information in the evaluation of neonatal ischemia and hypoxia[21]. Most authors now believe that vasoconstrictor ET-1 and diastolic factor adrenomedullin (ADM) are closely related to the development of neonatal pulmonary hypertension. The ET gene family includes ET-1, ET-2, ET-3 and ET-4, but human endothelial cells can only produce ET-1[22]. ET-1 binds to two main kinds of protein-coupled receptors, namely ETA and ETB[22]. When hypoxia occurs, the endothelial cells of lung tissue get damaged, and the expression and release of the ET-1 gene product increase. Once ET-1 concentrations in plasma and lung tissue are increased, ET-1 will bind to ET-1 receptors on pulmonary vascular smooth muscle and mediate vasoconstriction through phosphoinositide-mediated pathway-dependent processes[23], pulmonary hypertension will occur and become severer after combination with hypoxic pulmonary hypertension. ET-1 can directly promote activation of growth factors, and indirectly promotes the synthesis and secretion of some factors that further promote PASMC DNA synthesis and proliferation, leading to the process of pulmonary vascular remodeling[24]. *In vitro* investigations have shown that hypoxia can induce the formation of ADM[25]. Studies have found that ADM is increased in mice with hypoxia-induced pulmonary hypertension, and the ADM level is positively correlated with the degree of hypoxia[26]. Furthermore, the level of ADM mRNA increases in a time-dependent manner under hypoxia[26]. Zhao et al[26] also found in the rat model that pulmonary arterial pressure increases along with prolonged hypoxia. ADM levels in plasma and bronchoalveolar fluid lavage were significantly higher in the hypoxia groups than that in the control group and increased along with the pulmonary arterial pressure[27]. The mean pulmonary artery pressure (mPAP) decreases in rats after they are exposed to exogenous ADM, indicating that ADM is able to reduce mPAP to a certain extent[28]. Some scholars[28,29] have shown that hypoxia can promote the synthesis and secretion of ADM. Researches also found that ADM levels of patients with pulmonary heart disease are significantly higher than those in healthy patients, and correlated negatively with the PaO_2, indicating that hypoxia can stimulate ADM synthesis and secretion. Therefore, it can be considered that ET-1 and ADM act together on pulmonary vessels in hypoxic conditions, which may eventually lead to an increase in pulmonary artery pressure[29]. In this study, echocardiographic data were obtained from all participants. The results of analysis showed that only RVEF and tricuspid E/A ratio in the severe HPH group were significantly different (lower) from the same indicators in the control group at 1 d, 3 d and 7 d after birth. There were no significant differences in RVEF and tricuspid E/A ratio between the control group and the other HPH groups ($p > 0.05$), suggesting that they can only be used for the evaluation of ventricular function to diagnose the right ventricular systolic and diastolic dysfunction in the severe HPH group at 1 d, 3 d and 7 d after birth, which may be not applicable for patients with only mild or moderate HPH. The use of right ventricular Tei index for the evaluation of neonatal right ventricular function showed that the index in healthy neonates was 0.38 ± 0.09, and higher index values were found in neonates with HPH, indicating that right ventricular pressure is increased along with the increased pulmonary artery pressure. Therefore, the decline in the function of the whole right ventricle was found in all the three HPH groups. Right ventricular diastolic function was affected by pulmonary hypertension, the right ventricular pressure was increased, the tricuspid valve opening was delayed and the IRT was prolonged. At the same time, the right ventricular systolic force was reduced, so the increase in right ventricular systolic pressure slows down, and the pulmonary

valve opening is delayed, leading to the extension of ICT. The combined effect of reduced right ventricular systolic and diastolic function will lead to shortened ET and increase in the Tei index. While conventional methods can only detect the right ventricular dysfunction of severe HPH group, they are ineffective in non-severe HPH groups. The right ventricle Tei index, however, can be used to sensitively detect the existence of right ventricular dysfunction in mild and moderate groups, which provides strong evidence useful for prompt clinical diagnosis and treatment. Significant differences in HIF-1α level were found among the mild, moderate and severe HPH groups. Correlation analyses showed that HIF-1α and PASP were positively correlated, indicating that PASP levels are closely related to the serum HIF-1α level. Severe hypoxia always comes along with a high serum HIF-1α level and a high PASP value. So it can be hypothesized that hypoxia is an important factor increasing HIF-1α level and PASP. Clinical symptoms of hypoxia can be relieved by clinical treatment, thus HIF-1α levels and pulmonary artery pressure will also decrease. It was found in this study that hypoxia symptoms in mild and moderate HPH groups disappeared at 3 d after birth, HIF-1α also returned to the normal level at the same time point, but pulmonary artery pressure didn't return to the normal level until 7 days after birth. Hypoxia symptoms in the severe group disappeared at 7 d after birth, and HIF-1α also returned to the normal level at the same time point; however, pulmonary artery pressure did not return to the normal level, but decreased instead, indicating that the symptoms of hypoxia are relieved. HIF-1α degradation was probably accelerated and its level in serum was also decreased significantly. In addition, the decrease in serum levels came along with relieved hypoxia symptoms. It can be speculated that HIF-1α levels can be used to sensitively reflect the status of hypoxia conditions. It was found in our study that the changes in pulmonary artery pressure were not consistent with the hypoxic status or the change in serum HIF-1α level. Compared with remission from hypoxia and recovery of HIF-lα level, the recovery of pulmonary artery pressure lagged behind. A possible reason is that the pulmonary artery pressure is regulated by multiple factors under hypoxic conditions. For example, the function of downstream regulatory genes of HIF-1α does not change with the remission of hypoxia. But the specific mechanisms are still unknown. Due to perinatal hypoxia, the ET-1 level in the case group was increased along with the increased PASP at 1 d after birth, so the ET-1 level and PASP were positively correlated. With the improvement of hypoxic symptoms, the serum ET-1 level and pulmonary artery pressure also showed a downward trend. At 3 d after birth, hypoxia symptoms of mild and moderate groups and ET-1 levels of mild group were recovered, but PASP still did not return to the normal level. ET-1 level and PASP were positively correlated. At 7 d after birth, all the indicators of function except PASP and ET-1 levels in the severe group returned to normal levels, there was no correlation between ET-1 level and PASP; thus, ET-1, hypoxia and PASP are closely related. For 1-day-old neonates with hypoxia, the release of ET-1 was increased and resulted in an enhanced response of pulmonary vascular to ET-1, leading to small blood vessels contraction, and significantly increased PASP. In these cases the higher pulmonary artery pressure always came along with higher serum ET-1 levels. Although hypoxia conditions were corrected at 3 d after birth, PASP was still increasing; also, ET-1 in the moderate group still did not return to the normal level. The possible explanation is that hypoxia conditions of the mild HPH group at this time point were corrected and led to a decreased release of ET-1, but pulmonary artery pressure regulated by various vasoconstrictor factors still did not return to the normal level. Although the hypoxia symptoms in the moderate group were improved, the existing damage to the vascular endothelial cells could still cause the abnormal release of ET-1 into the blood. In turn, the strong vasoconstriction function of ET caused continuous small pulmonary artery spasm, resulting in the increase in PASP. For the same reason, ET-1 levels and PASP in the severe group were increased significantly even at 7 d after birth. Data in this study showed a positive correlation between the changes of ET-1 and PASP, indicating that ET-1 may play an important role in the pathogenesis of HPH and can also reflect the changes in pulmonary artery pressure to a considerable degree. Same scholars have found that the ET-1 content in the rats treated with hypoxic conditions for 28 days was higher than that in the rats in hypoxic environment for only 14 d, and the ET-1 content was positively correlated to the pulmonary arterial pressure, suggesting that ET-1 may be an important factor in hypoxic pulmonary vasoconstriction and pulmonary hypertension. In this report, the degree of changes in the serum ADM level in the three HPH groups

was higher than that in the ET-1 level. One possible reason is the selective expression repression caused by hypoxia, but no relevant experimental data is available to sustain this claim. It is worth noting that the HIF-1α levels in patients with HPH was recovered faster than ET-1 level and pulmonary artery pressure, so clinicians who treat patients with pulmonary hypertension should note that the blood vessel dilation should be performed after improvement of hypoxic symptoms to balance the vasoconstriction due to ET-1.

Conclusions

We observed that the PASP of patients with HPH was correlated with the serum HIF-lα, ET-1 and ADM levels. Hypoxia can increase the expression level of HIF-1α, and further increase the expression of its downstream target genes ET-1 and ADM, eventually leading to pulmonary hypertension. Echocardiography showed that the decreases of the RVEF and tricuspid E/A ratio were only apparent in the severe HPH group, but the right ventricular Tei index could sensitively reflect the right ventricular dysfunction in mild, moderate and severe HPH patients at an early stage. The ultrasonic method can be used in clinical application to monitor the changes in pulmonary artery pressure and right ventricular function, providing vital information for early treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Esquinas RA, Ioannis P. A link of non-invasive ventilation in chronic hypercapnic COPD patients with pulmonary hypertension. A new index of prognosis? Eur Rev Med Pharmacol Sci 2012; 16: 1133-1134, 1135-1136.
2) Drummond WH. Neonatal pulmonary hypertension. Equine Vet J 1987; 19: 169-171.
3) Das R, Balonan L, Ballard HJ, Ho S. Chronic hypoxia inhibits the antihypertensive effect of melatonin on pulmonary artery. Int J Cardiol 2008; 126: 340-345.
4) Greenough A, Khetriwal B. Pulmonary hypertension in the newborn. Paediatr Respir Rev 2005; 6: 111-116.
5) Wedgwood S, Lakshminrusimha S, Schumacker PT, Steinhorn RH. Hypoxia inducible factor signaling and experimental persistent pulmonary hypertension of the newborn. Front Pharmacol 2015; 6: 47.
6) Preston IR. Clinical perspective of hypoxia-mediated pulmonary hypertension. Antioxid Redox Signal 2007; 9: 711-721.
7) Alapati D, Jassar R, Shaffer TH. Management of supplemental oxygen for infants with persistent pulmonary hypertension of newborn: a survey. Am J Perinatol 2017; 34: 276-282.
8) Gagnon MH, Wintermark P. Effect of persistent pulmonary hypertension on brain oxygenation in asphyxiated term newborns treated with hypothermia. J Matern Fetal Neonatal Med 2016; 29: 2049-2055.
9) Malowitz JR, Forsha DE, Smith PB, Cotten CM, Barker PC, Tatum GH. Right ventricular echocardiographic indices predict poor outcomes in infants with persistent pulmonary hypertension of the newborn. Eur Heart J Cardiovasc Imaging 2015; 16: 1224-1231.
10) Aggarwal S, Natarajan G. Echocardiographic correlates of persistent pulmonary hypertension of the newborn. Early Hum Dev 2015; 91: 285-289.
11) Saini-Chohan HK, Dakshinamurti S, Taylor WA, Shen GX, Murphy R, Sparagna GC, Hatch GM. Persistent pulmonary hypertension results in reduced tetralinoleoyl-cardiolipin and mitochondrial complex II + III during the development of right ventricular hypertrophy in the neonatal pig heart. Am J Physiol Heart Circ Physiol 2011; 301: H1415-H1424.
12) Sernich S, Carrasquero N, Lavie CJ, Chambers R, McGettigan M. Noninvasive assessment of the right and left ventricular function in neonates with congenital diaphragmatic hernia with persistent pulmonary hypertension before and after surgical repair. Ochsner J 2006; 6: 48-53.
13) Abraham S, Weismann CG. Left ventricular End-Systolic eccentricity index for assessment of pulmonary hypertension in infants. Echocardiography 2016; 33: 910-915.
14) James AT, Corcoran JD, McNamara PJ, Franklin O, El-Khuffash AF. The effect of milrinone on right and left ventricular function when used as a rescue therapy for term infants with pulmonary hypertension. Cardiol Young 2016; 26: 90-99.
15) Oishi P, Datar SA, Fineman JR. Advances in the management of pediatric pulmonary hypertension. Respir Care 2011; 56: 1314-1339.
16) Sugiura T, Suzuki S, Hussein MH, Kato T, Togari H. Usefulness of a new Doppler index for assessing both ventricular functions and pulmonary circulation in newborn piglet with hypoxic pulmonary hypertension. Pediatr Res 2003; 53: 927-932.
17) Sugiura T, Suzuki S, Hussein MH, Kato T, Okubo Y, Imamine H, Sugiura T, Togari H. The Tei index permits evaluation of cardiopulmonary function during inhaled nitric oxide therapy in the hypoxic newborn piglet. Biol Neonate 2004; 86: 176-182.

18) Lemus-Varela ML, Flores-Soto ME, Cervantes-Munguia R, Torres-Mendoza BM, Gudino-Cabrera G, Chaparro-Huerta V, Ortuno-Sahagun D, Beas-Zarate C. Expression of HIF-1 alpha, VEGF and EPO in peripheral blood from patients with two cardiac abnormalities associated with hypoxia. Clin Biochem 2010; 43: 234-239.

19) Cohen SS, Powers BR, Lerch-Gaggl A, Teng RJ, Konduri GG. Impaired cerebral angiogenesis in the fetal lamb model of persistent pulmonary hypertension. Int J Dev Neurosci 2014; 38: 113-118.

20) Wedgwood S, Lakshminrusimha S, Schumacker PT, Steinhorn RH. Hypoxia inducible factor signaling and experimental persistent pulmonary hypertension of the newborn. Front Pharmacol 2015; 6: 47.

21) Nakanishi K, Osada H, Uenoyama M, Kanazawa F, Ohrui N, Masaki Y, Hayashi T, Kanatani Y, Ikeda T, Kawai T. Expressions of adrenomedullin mRNA and protein in rats with hypobaric hypoxia-induced pulmonary hypertension. Am J Physiol Heart Circ Physiol 2004; 286: H2159-H2168.

22) Therese P. Persistent pulmonary hypertension of the newborn. Paediatr Respir Rev 2006; 7 Suppl 1: S175-S176.

23) Gien J, Tseng N, Seedorf G, Roe G, Abman SH. Endothelin-1 impairs angiogenesis in vitro through Rho-kinase activation after chronic intrauterine pulmonary hypertension in fetal sheep. Pediatr Res 2013; 73: 252-262.

24) Wolf D, Tseng N, Seedorf G, Roe G, Abman SH, Gien J. Endothelin-1 decreases endothelial PPAR-gamma signaling and impairs angiogenesis after chronic intrauterine pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2014; 306: L361-L371.

25) Vizza CD, Letizia C, Badagliacca R, Poscia R, Pezzuto B, Gambardella C, Nona A, Papa S, Marcon S, Mancone M, Iacoboni C, Riccieri V, Volterrani M, Fedele F. Relationship between baseline ET-1 plasma levels and outcome in patients with idiopathic pulmonary hypertension treated with bosentan. Int J Cardiol 2013; 167: 220-224.

26) Zhao CF, Wang LJ, Gao L, Chang P, Xia W, Wang R, Sun RP. [Roles and signal pathways of adrenomedullin and adrenotensin in pulmonary remodeling due to left to right shunt in rats]. Zhonghua Er Ke Za Zhi 2008; 46: 585-590.

27) Ertmer C, Morelli A, Rehberg S, Lange M, Hucklenbruch C, Van Aken H, Booke M, Westphal M. Exogenous adrenomedullin prevents and reverses hypodynamic circulation and pulmonary hypertension in ovine endotoxaemia. Br J Anaesth 2007; 99: 830-836.

28) Gong YS, Fan XF, Wu XM, Hu LG, Tang CS, Pang YZ, Qi YF. [Changes of intermedin/adrenomedullin 2 and its receptors in the right ventricle of rats with chronic hypoxic pulmonary hypertension]. Sheng Li Xue Bao 2007; 59: 210-214.

29) Kamata S, Kamiyama M, Usui N, Kitayama Y, Okuyama H, Kubota A, Kangawa K. Is adrenomedullin involved in the pathophysiology of persistent pulmonary hypertension of the newborn? Pediatr Surg Int 2004; 20: 24-26.