Revista Argentina de Clínica Psicológica
2020, Vol. XXIX, N°5, 1281-1285
DOI: 10.24205/03276716.2020.1123



# The expression and clinical significance of serum miR-424, miR-205 and miR-155 in neonates with asphyxia

**Juan Yang[a*], Yanan Guo[b]**

**Abstract**

**Objectives:** To detect the expression levels of miR-424, miR-205 and miR-155 in the serum of newborns with asphyxia, so as to provide scientific basis for the early diagnosis and treatment of the above mirnas in the serum in neonatal asphyxia.

**Methods:** The expression levels of miR-424, miR-205 and miR-155 in serum of 110 newborns with asphyxia were detected by RT-QPCR and analyzed statistically. The correlation between miRNA and the severity of asphyxia was analyzed.

**Results**: There were no statistically significant differences in gender, gestational age, birth quality, etiology, maternal age, twins, cesarean section and amniotic fluid abnormalities between mild asphyxia group (survival) and severe asphyxia group (death) ($P > 0.05$). There was statistical difference in the scores of Apagar for 1min and 5min between the two groups ($P < 0.05$), while there was no statistical difference in the scores of apagar for 10min ($P > 0.05$). All the 3 miRNA could be detected in the serum of asphyxiated newborns, and compared with the control group, the expressions of miR-424 and miR-155 were up-regulated and the expressions of MiR-205 were down-regulated, with statistically significant differences.

**Conclusions:** the expression of miR-424 and miR-155 in the serum of children with asphyxia is up-regulated, which is positively correlated with the severity of the disease, while the expression of miR-205 is down-regulated, which is negatively correlated with the severity of the disease. Therefore, detection of miRNA expression in the serum of newborns with asphyxia has very important clinical value for early diagnosis and prognosis judgment of newborn asphyxia.

**Keywords:** suffocation; The miRNA expression; Clinical significance; serum

## Backgrounds

Neonatal asphyxia is an obstetric complication caused by hypoxia of the fetus in utero or during delivery, with respiratory disorders as the main manifestation. It is an emergency of the newborn. Effective improvement of the success rate of neonatal resuscitation and reduction of complications after asphyxiation resuscitation are important links in reducing neonatal mortality [1]. Hypoxic ischemia is the most common cause of severe neurological diseases caused by brain injury in neonates, with serious illness and high mortality. Some of the children survived with cerebral palsy (cerebral palsy), mental retardation and other sequelae. Correct and effective treatment of hypoxic ischemic brain injury (HIBD) is of great significance for reducing perinatal mortality, reducing disability rate and improving quality of life. It is an important issue for neonatal medicine to find an early indicator to predict neonatal hypoxic-ischemic brain injury. Micrornas (mirnas) are a newly discovered class of single-stranded non-coding small molecular Rnas. By binding to the target site, target mRNA or inhibitory protein translation can be degraded rapidly and effectively. With the deepening of its research, miRNA is found to be involved in almost all physiological and pathological processes and plays an important regulatory role. Several studies have reported using serum miRNAs as molecular markers for early prediction of disease.

miRNAs are a group of small non-coding nucleotides with a length of 18-25 nucleotides, which participate in the post-transcriptional regulation of target genes by means of miRNA shear and protein translation inhibition, and play an important role in cell differentiation, proliferation, apoptosis, angiogenesis and inflammatory immune

*[a] Department of Child Health,The Fourth Hospital of Shijiazhuang Shijiazhuang City, Hebei Province 050000, China*
*[b] Department of Pathogen Biology, Chongqing Medical University, Chongqing City 400016, China*
**Corresponding Author: Juan Yang Address: Department of Child Health,The Fourth Hospital of Shijiazhuang Shijiazhuang City, Hebei Province 050000, Chin Email: jingshu8215@163.com*

response. Studies have shown that miRNA is involved in the process of neonatal respiratory distress and plays an important regulatory role in its inflammatory response and apoptosis, which is expected to be a new biomarker and a new therapeutic target for neonatal asphyxia [2,3]. Other studies have shown that miRNA expression changes occur during neonatal asphyxia, and miRNA is involved in the pathophysiological process of epithelial cells, endothelial cells and macrophages, which determines the course of disease and prognosis of patients. Recent studies have shown that mir-155 is differentially expressed in acute knowledge distress syndrome associated with neonatal asphyxia, and it is expected to be a potential target for the treatment of asphyxia-related diseases by regulating pathways and immune responses by affecting the expression of its target genes [4,5].

In this study, the expression of miR-424, miR-205 and miR-155 genes in the serum of neonatal asphyxia patients was detected to explore the clinical significance of their expression levels in the early diagnosis and prognosis of neonatal asphyxia.

**Materials and Methods**

**1.1 Research Objects**

The cases were from 100 asphyxiated newborns admitted to the Neonatal Department of our hospital Hospital from September 2018 to September 2020, including 60 males and 40 females. Gestational age is 37 ~ 42 weeks. Birth weight: 2000-4000 g. The clinical manifestations and classification were consistent with the diagnostic criteria of neonatal asphyxia. Apgar score at birth was used to judge: Apgar score at 1 min at birth ranged from 0 to 3 as severe asphyxia, and from 4 to 7 as mild asphyxia. If Apgar score at birth ranged from 8 to 10, but the score was less than 7 after several minutes, it was also considered asphyxia group. There were 75 cases of mild asphyxia and 25 cases of severe asphyxia. There were no statistically significant differences between the control group and the observation group in terms of gestational age, gender, weight at birth, delivery mode and other general conditions (P>0.05).

**1.2 Main reagents and instruments**

Trizol (TRI Reagent BD), Taqman microRNA Reverse Transciption Kit, Taqman microRNA Assay and Taqman 2X Universal PCR Master Mix II, purchased from Applied Biosystems ABI.

**1.3 Detection method**

2 ~ 3 ml of venous blood in the acute stage (within 3 days after birth) of children in the asphyxia group was extracted. In normal control group, 3 ml umbilical cord blood was collected after birth and injected into heparin anticoagulant tube. The serum was centrifuged at 3000 r/min for 10 min, and then separated into 1.5M1 EP tubes, which were stored in a refrigerator at -70℃ for centralized detection. 1.3.1 Extraction of total RNA: Serum was dissolved at room temperature, 250ul serum was taken and put into 1-5 M1 EP tube, 750ul Trizol was added, shaken and mixed, and placed at room temperature for 5 minutes. Add 200ul chloroform into the EP tube and shake violently for 15 s. It was placed at room temperature for 5 min and centrifuged at 12000 g for 15 min at 4℃. The upper aqueous phase was carefully absorbed into another EP tube, isopropyl alcohol of the same volume was added, gently reversed for 5 times, stood for 5min, centrifuged at 4℃ for 8 min at 12,000 g. Supernatant was discarded, and 75 M1 / dL ethanol 1 M1 was added to the precipitation, which was vortex, stood for 2 min, centrifuged at 4℃ for 7 500 g for 5 min. The supernatant was discarded, the ultra-clean table was air-dried at room temperature, 20ul enzyme-free water was added, blown, RNA precipitation was fully dissolved, and the total RNA was transferred into the 200ul PCR tube. RNA mass and concentration were determined by a 2UL UV spectrophotometer.

**1.3.2 RT PCR**

RT reaction system 15UL: 3 p1RTprime, 0.15ul 100 mmol/L dNTPs (with DTTP), 1.00ul MultiScriber Reverse Transcriptase(50U/ul), 1.50ul 10× Reverseinhibiting Buffer, 0.19ul RNase Inhibitor(20U/ul), 4.16ul Nuclease free water, 5.00 ul totalRNA. Reaction conditions: 30 min at 16℃, 30 min at 42℃, and 5 min at 85℃. PCR system 20ul: 10ul TaqMan 2×Universal PCR Master MixII(no UNG), 7.67ul Nuclease free water, 1ul TaqManr MicroRNA Assays, 1.33ul reverse transcription products.

Reaction conditions: 10 min at 95 ° C, 15 s at 95 ° C and 1 min at 60 ° C, followed by 2 steps for 40 cycles. The mRNA expression of the target gene was determined by Ct value, and the relative change of gene expression was expressed by 2-△ Ct.

**1.4 Statistical analysis**

All data were processed with SPSS 22.0 statistical software package. Measurement data were expressed as mean ± standard deviation (x±s), and comparison between groups was performed by T test. P<0.05 was considered statistically significant.

**Results**

1. General information of newborns of the two groups

Gender, gestational age, birth quality, etiology, mother's age, twins, cesarean section and abnormal amniotic fluid were not significantly different between the mild asphyxia group (survival) and the severe asphyxia group (death) of newborns with asphyxia (P > 0.05), and the results were shown in table 1.

*Table 1.* **Comparison of general data of neonatal asphyxia group and severe asphyxia group**

| Project | Mild asphyxia group | Severe asphyxia group | X2/t value | P value |
|---|---|---|---|---|
| Male （%） | 65% | 68% | 0.26 | 0.36 |
| Gestational age (X ± S, weeks) | 37.21±1.16 | 37.69±1.25 | 0.58 | 0.56 |
| Birth mass (x±s, g) | 2863±208 | 2869±211 | 0.25 | 0.69 |
| Etiology (%) | | | 0.23 | 0.21 |
| choking | 38.3% | 35.2% | | |
| Meconium aspiration syndrome | 24.8% | 29.6% | | |
| pneumonia | 21.3% | 18.9% | | |
| sepsis | 18.5% | 10.2% | | |
| Mother's age (x±s, age) | 28.6±3.65 | 29.8±4.32 | 1.19 | 0.25 |
| Twin (%) | 6.5% | 8.9% | 0.26 | 0.63 |
| Cesarean delivery | 61.2% | 68.9% | 0.32 | 0.65 |
| Amniotic fluid anomaly | 8.5% | 11.9% | 0.59 | 0.56 |

**2. Apgar score of newborns in the two groups**

There were no statistically significant differences in gender, gestational age, birth weight and delivery mode between the two groups (P > 0.05), indicating that the two groups were comparable. There were statistically significant differences in the scores of apafar for 1min and 5min between the two groups (P < 0.05), while there was no statistical difference in the scores of apafar for 10min (P > 0.05), as shown in table 2.

*Table 2.* **Apgar score at birth in both groups**

| ApgarValue | Severe asphyxia group | Mild asphyxia group | T value | P value |
|---|---|---|---|---|
| 1min Apgar | 5.23±1.89 | 8.22±2.15 | 5.89 | 0.00 |
| 5min Apgar | 7.56±2.16 | 9.23±2.25 | 2.15 | 0.01 |
| 10min Apgar | 9.23±3.05 | 9.98±3.65 | 0.85 | 0.45 |

**3. Serum miRNA expression levels in newborns with asphyxia with different prognosis**

The expression levels of miR-424, miR-205 and miR-155 in the serum of newborns with asphyxia showed that all the three mirnas could be detected in the serum of newborns with asphyxia. Moreover, compared with the control group, the expressions of miR-424 and miR-155 were up-regulated and the expressions of miR-205 were down-regulated, with statistically significant differences (P < 0.05). See Table 3 for details.

*Table 3.* **Comparison of the expression levels and NCIS score of serum miR-424, miR-205 and miR-155 in neonatal asphyxia group and normal group (X ± S)**

| Group | n | miR-424 | miR-205 | miR-155 | NCIS value |
|---|---|---|---|---|---|
| Severe asphyxia group | 75 | 1.68±0.24 | 0.35±0.09 | 2.54±0.65 | 86.65±3.25 |
| Mild asphyxia group | 25 | 0.86±0.12 | 1.79±0.21 | 0.59±0.05 | 81.36±3.8 |
| t Value | | 13.25 | 15.46 | 10.25 | 9.85 |
| P Value | | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

**Conclusions**

The essence of neonatal asphyxia is the organic or functional damage of organs caused by anoxic acidosis, among which brain and heart are the most sensitive organs to anoxia. At present, Apgar score is still an internationally recognized simple and effective method to evaluate the existence and extent of neonatal asphyxia. Apgar score is used to judge the existence and extent of neonatal asphyxia based on the respiration, heart rate, muscle tone, skin color and responsiveness to stimulation of newborn [6]. But Apgar score is affected by many

factors, such as pregnant women used analgesic sedatives, fetal medical staff in the process of low muscle tone, score, subjective factors such as experience and psychology, so the Apgar score of the sensitivity of the diagnosis of asphyxia, specific degrees are not strong, and easy to misdiagnosis or missed diagnosis, so it is necessary to seek a kind of specific objective indicators. With the development of the human post-genome project, the non-coding sequences accounting for 99% of the human genome have attracted increasing attention, among which the discovery of miRNA is the most remarkable. Mirnas are a class of endogenous non-coding single-stranded RNA molecules, most of which are between 18 and 25 nucleotides in length, and widely exist in viruses, plants and higher mammals. It plays an important role in a variety of physiological and pathological processes, such as cell proliferation and differentiation, tissue development, glycolipid metabolism, inflammation, immune response and tumorigenesis [7,8]. According to literature reports, the expression level of miR-155 in patients with acute lung injury was significantly upregulation, and miR-155 has certain value in predicting the 30-day mortality rate of patients with acute lung injury, and it can be used as a biomarker to evaluate the prognosis of patients with acute lung injury.

A large number of recent studies have shown that mirnas can be detected in the serum with stable expression, and there are different miRNA expression profiles in the serum of patients with different diseases, which can be used as molecular markers for early prediction of diseases. So, are there specific mirnas in children with neonatal asphyxia that can be used to predict the risk of neonatal asphyxia early? Of this study is to begin with, the selection has been study in ischemic anoxia of miRNAs in expression in cells as the research object, clear whether stable expression in serum, and presence of expression differences, and the results show that three kinds of miRNA expression can be detected in all groups, miR - 424, miR - 155 in children with asphyxia expression level in serum and can be used as molecular markers of early prediction ischemia anoxic encephalopathy. The maintenance of oxygen homeostasis is the basis for the growth and development of aerobic organisms.

Gender, gestational age, birth quality, etiology, maternal age, twins, cesarean section and abnormal amniotic fluid were not significantly different between the mild asphyxia group (survival) and the severe asphyxia group (death) ($P > 0.05$). There was statistical difference in the scores of apafar for 1min and 5min between the two groups ($P < 0.05$), while there was no statistical difference in the scores of apafar for 10min ($P > 0.05$). All the 3 mirnas could be detected in the serum of asphyxiated newborns, and compared with the control group, the expressions of miR-424 and miR-155 were up-regulated and the expressions of miR-205 were down-regulated, with statistically significant differences.

Multiple studies have confirmed that miRNA can rapidly and reversibly regulate the expression of target genes through the classic hypoxiainduced factor 1A (HIF1a) pathway and the HIF 1A independent pathway, and participate in the repair process of central nervous system (CNs) injury after hypoxic ischemia, playing an important role in the diagnosis and treatment of hypoxic ischemic brain injury (HIBD). miR-424, miR-155, etc., are the major Non-hiF-1A dependent mirnas, among which miR-205 has been confirmed as the major anti-apoptotic factor. miR-424 and miR-155 are expressed in any ischemic anoxic cells. Microrna-424 (mir-424) has a protective effect on ischemic injury of brain tissue caused by ACI [10,11]. However, there are few reports on the role of serum circulating miR-424 in the early diagnosis and prognosis of ACI. According to a study of post-perinatal miRNS fever in neonates, miRNA-155 is significantly upregitated in neonates with ischemic encephalopathy [12]. Its related research may be a new target for the treatment of cerebral ischemic diseases.

The expressions of miR-424 and miR-155 in the serum of children with asphyxia were up-regulated and positively correlated with the severity of the disease, while the expressions of miR-205 were down-regulated and negatively correlated with the severity of the disease. Therefore, detection of miRNA expression in the serum of newborns with asphyxia has very important clinical value for early diagnosis and prognosis judgment of newborn asphyxia.

Although there are multiple miRNA expressions in the ischemic anoxic cells of asphyxiated newborns, the detection of miRNAs in the blood of infants with ischemic anoxia showed in this study that not all of these miRNA expressions were up-regulated in the blood, and some miRNA expressions were not different. The specific reasons are not clear. These Up-regulated mirnas are expected to be used as specific molecular markers for early prediction of ischemic and hypoxic encephalopathy.

**Funding**

The work was supported by the key sci-

technological research of Hebei Province Health Department
No. 20120230.

**Reference**

[1] Solevåg AL, Schmölzer GM, Cheung PY. Novel interventions to reduce oxidative-stress related brain injury in neonatal asphyxia. Free Radic Biol Med. 2019 Oct; 142:113-122.
[2] Simonson B, Das S. MicroRNA Therapeutics: The Next Magic Bullet? Mini Rev Med Chem. 2015;15(6):467-74.
[3] Vishnoi A, Rani S. MiRNA Biogenesis and Regulation of Diseases: An Overview. Methods Mol Biol. 2017; 1509:1-10.
[4] Michaille JJ, Awad H, Fortman EC, Efanov AA, Tili E. miR-155 expression in antitumor immunity: The higher the better? Genes Chromosomes Cancer. 2019 Apr;58(4):208-218.
[5] Jiang J, Song Z, Zhang L. miR-155-5p Promotes Progression of Acute Respiratory Distress Syndrome by Inhibiting Differentiation of Bone Marrow Mesenchymal Stem Cells to Alveolar Type II Epithelial Cells. Med Sci Monit. 2018 Jun 24; 24:4330-4338.
[6] Witcher TJ, Jurdi S, Kumar V, Gupta A, Moores RR Jr, Khoury J, Rozycki HJ. Neonatal Resuscitation and Adaptation Score vs Apgar: newborn assessment and predictive ability. J Perinatol. 2018 Nov;38(11):1476-1482.
[7] Friedman JM, Jones PA. MicroRNAs: critical mediators of differentiation, development and disease. Swiss Med Wkly. 2009 Aug 22;139(33-34):466-72.
[8] Nelson KB, Emery ES 3rd. Birth asphyxia and the neonatal brain: what do we know and when do we know it? Clin Perinatol. 1993 Jun;20(2):327-44.
[9] Duan Baozhen, Wang Xiangzhen, Chen Jun. Correlation between HSP70 and HIF-1a levels in cord blood of neonates with hypoxic-ischemic encephalopathy[J]. Asia-Pacific Traditional Medicine, 2017(13):119-121.
[10] Zhao H, Wang J, Gao L, et al. MiRNA-424 protects against permanent focal cerebral ischemia injury in mice involving suppressing microglia activation J］. Stroke，2013，44（6）：1706-1713.
[11] Ponnusamy V, Kapellou O, Yip E, Evanson J, Wong LF, Michael-Titus A, Yip PK, Shah DK. A study of microRNAs from dried blood spots in newborns after perinatal asphyxia: a simple and feasible biosampling method. Pediatr Res. 2016 May;79(5):799-805.
[12] Ponnusamy V, Kapellou O, Yip E, Evanson J, Wong LF, Michael-Titus A, Yip PK, Shah DK. A study of microRNAs from dried blood spots in newborns after perinatal asphyxia: a simple and feasible biosampling method. Pediatr Res. 2016 May;79(5):799-805.