

Role of microRNA-498 and microRNA-410 in neonatal hypoxic-ischemic encephalopathy

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Background: Neonatal asphyxia is the primary cause of hypoxic-ischemic encephalopathy (HIE), a condition characterized by hypoxic and ischemic brain damage. A class of short noncoding RNAs known as microRNAs (miRNAs) have significant regulatory functions, can function as diagnostic and developmental indicators of diseases, and are involved in disease pathophysiology.

Purpose: To study the role of microRNA-410 and microRNA-498 in neonatal HIE as well as control and prevention of neonatal encephalopathy.

Methods: A case-control study was performed on 30 full-term neonates with proven HIE, and 30 clinically healthy full-term neonates with no evidence of HIE matched for age and sex serving as controls. The expression of microRNA-498 and microRNA-410 were measured using quantitative real-time polymerase chain reaction.

Results: Levels of miRNA-410 and miRNA-498 were higher in cases versus controls (1.56 ± 6.43 copies/mL vs 0.58 ± 0.60 copies/mL) and (55.63 ± 118.24 copies/mL vs 3.74 ± 7.09 copies/mL), respectively. Of the cases, 66.7% were discharged cases and 33.3% died. Overall miRNA-410 had a sensitivity of 70%, specificity of 60%, and cutoff point of ≤ 0.242 , whereas miRNA-498 had a sensitivity of 70%, specificity of 66.7%, and cutoff point > 1.854 .

Conclusion: These findings suggest that miRNA-498 and miRNA-410 can be auxiliary diagnostic and prognostic tools for neonatal HIE.

Key words: MicroRNA-498, MicroRNA-410, Neonatal hypoxic-ischemic encephalopathy.

Findings: miRNA-498 and miRNA-410 can be auxiliary diagnostic and prognostic tools for neonatal HIE.

Meaning: we can use miRNA-498 and miRNA-410 as markers and indicator for HIE.

Introduction

Neonatal asphyxia is the primary cause of neonatal hypoxic-ischemic encephalopathy (HIE), a condition characterized by hypoxic and ischemic brain damage.¹ For newborns afflicted, it is a primary cause of neurological problems and permanent impairment.² The prevalence of HIE is 1–8 for every 1,000 live births in affluent nations, while it is 26 for every 1,000 live births in underdeveloped countries.³ The conventional therapy for hypothermia (HIE) in high-income nations, induced hypothermia, is less successful among low-income populations, who have the largest burden of illness.⁴ Thus, for neonates with HIE, early identification and effective treatment strategies are therefore critically essential.

A class of tiny noncoding RNAs known as microRNAs (miRNAs) have significant regulatory effects on a broad range of biological activities, including cell division, invasion, migration, and proliferation.⁵ Emerging data indicates that dysregulation of miRNAs is typically present in human illnesses.⁶ These abnormal miRNAs may both accelerate the onset of illness and serve as biomarkers for the detection and treatment of illness.⁷ Additionally, functional miRNAs, such as miRNA-210, have been discovered to be essential components in the development and progression of neonatal HIE,⁸ also, miRNA-17-5p,⁹ and miRNA-204.¹⁰

In a recent research, O'Sullivan examined the changes in

Key message

Question: Is it role of microRNA-410 (miRNA-410) and microRNA-498 (miRNA-498) in neonatal hypoxic-ischemic encephalopathy (HIE)?

miRNA expression in umbilical cord blood from newborns suffering from HIE. In entire blood samples, one of the dysregulated miRNAs was miRNA-410.¹¹⁾ Furthermore, miRNA-410 has been shown to play a protective effect in Parkinson disease against brain damage,¹²⁾ and anesthesia-induced cognitive dysfunction.¹³⁾ The biological role and clinical importance of miR-410 in newborn HIE, however, are still completely unclear.

Numerous investigations aimed to assess expression level and diagnostic efficacy of miRNA-410 in neonates with HIE to enhance the diagnosis process. Furthermore, Su et al.¹³⁾ created an oxygen and glucose deprivation (OGD)-induced cell damage model in PC12 and SH-SY5Y cells, which is the most widely used *in vitro* model for HIE to detect the biological function of miR-410 in controlling neuronal cells' survival.¹⁴⁾

Meng et al.¹⁴⁾ demonstrated that serum miRNA-410 expression was considerably lower in OGD-injured cell models and HIE neonates. The upregulation of miRNA-410 in PC12 and SH-SY5S cells reversed the effects of OGD-induced reduced cell viability, increased apoptosis of the cells and triggered neuroinflammation. Phosphatase and tensin homolog (PTEN) was suggested as miRNA-410's immediate target. It was shown that miRNA-410 was deregulated in serum in HIE and it functions as a potential indicator for diagnosis and improves vitality of cells in OGD-damaged cells by blocking cellular death via targeting PTEN. Thus, this study aimed to study the role of microRNA-498 and microRNA-410 in neonatal HIE and their role in controlling and prevention of neonatal encephalopathy.

Methods

This study was done with the cooperation between the Medical Biochemistry and Molecular Biology department and the neonatal intensive care unit (NICU) of Menoufia University Hospitals.

The Menoufia University Ethics Committee approved the study, which complies with the Helsinki Declaration (IRB:12/2023 PEDI 116). Prior to their enrollment, participants provided written consent.

1. Study design

This work included 60 neonates divided into 2 groups: group 1, 30 full-term neonates with HIE; group 2, 30 clinically healthy full-term neonates without any proof of neonatal HIE; matched for age and sex with the cases.

2. Inclusion criteria

1) **Study group:** Both sex full-term newborns exhibiting signs and symptoms of neonatal HIE based on clinical Sarnat staging.¹⁵⁾

2) **Control group:** Full-term neonates without any signs of HIE.

3. Exclusion criteria

We excluded neonates with congenital infection, probable inborn metabolic problems, perinatal hypoxia, congenital malformations, chromosomal abnormalities, pre-term neonates, infant born to diabetic mothers.

Prior to the collection of blood samples, all parents provided written agreement, which was approved by the Menoufia University research ethics and human rights commission.

Every patient was exposed to taking a thorough history including prenatal, natal and postnatal history, comprehensive clinical assessment with special focusing on clinical signs of HIE in the form of: (1) respiratory dysfunction, including retracted intercostal, apnea, and elevated oxygen needs. (2) circulatory dysfunction including inadequate peripheral circulation, hypotension, tachycardia, shock and extended capillary refill. (3) Using the modified Sarnat score, the degree of encephalopathy, if any, will be determined 24 hours after delivery. (4) Additionally, on day 3 and at discharge, standardized neurologic examination was carried out.

Routine investigations include complete blood count (CBC), C-reactive protein (CRP), liver and kidney function tests.

4. Specific investigations

Assessment of serum miRNA-410 and miRNA-498 levels by quantitative real-time polymerase chain analysis (qRT-PCR).

5. Blood sample collection

Umbilical cord blood samples were obtained from all neonates in this study; The Tempus Blood RNA tubes were filled with 3 mL of cord blood and bio banked at -80C till RNA extraction.

6. RT-PCR

1) RNA extraction

With a miRNeasy kit (QIAGEN), total RNA including miRNAs was extracted. Once purification of microRNA the quantified by NanoDrop N50 nanophotometer Implen GmbH and Implen, Inc. Schatzbogen 5281829 Munich, Ger-

many by measuring the ratio of absorbance at 260/280 nm to estimate both RNA concentration and purity. The RNA extract was stored at -80°C.

2) Reverse transcription and cDNA production

Using the miScript II RT kit (QIAGEN), reverse transcription was used to produce cDNA. Twenty microliter of reaction mixture (4 µL of miScript HiSpec RT buffer, 2 µL of miScript Nucleics Mix, 2 µL of miScript™ reverse transcriptases, 2 µL of nuclease-free H₂O, and 10 µL of pure miRNA) were used to complete the reaction on ice. In order to suppress the reverse transcriptase enzyme, the reaction was preceded by one cycle of 37°C for 60 minutes and then 95°C for 5 minutes in a 2720 Applied Bio-systems thermal cycler (Singapore). The cDNA that was produced was stored at -20°C until the real-time PCR phase. Using a miScript SYBR Green PCR kit (QIAGEN).

3) Real-time PCR

Real-time PCR was performed. cDNA was diluted 1:5 with nuclease-free H₂O prior to reaction processing, and a net volume of 25 µL (12.5 µL of SYBR Green Master Mix, 3.5 µL of nuclease-free water, 4 µL of diluted cDNA, 2.5 µL of miScript universal primer, and 2.5 µL of miScript primer assay) was employed. As a reference gene, U6 was amplified for normalization. The following primers were used; mature miR-410 AGGUUGUCUGUGAUGAGUUCG and miR 498: UUUCAAGCCAGGGGCGUUUUUC and as a reference gene, U6 was amplified for normalization (mi Script primer assay kit, QIAGEN) Forward primer: GTGCTCGCTTCGGCAGCA Reverse primer CAAAATATGGAACGCTTC

Analysis of data was accomplished Using the ABI7500 Real-Time PCR apparatus with software version 2.0.1 and the subsequent cycle parameters: The first activation stage took place for 15 minutes at 95°C followed by 40 cycles of 3-step cycling of denaturation at 94°C for 15 seconds then, for 30 seconds, primer annealing at 55°C, followed by primer extension at 70 °C for 30 seconds. miR-410 and miR-498 expression levels were compared to U6 expression levels, and relative expression values computed with the 2- $\Delta\Delta C_t$ technique. Melting curve analysis was used to verify the specificity of the amplification and the lack of primer dimers for each run.

6. Statistical analysis

The computer was given data, and IBM SPSS Statistics ver. 22.0 (IBM Co.). Numbers and percentages were used to describe the qualitative data. To confirm that the distribution was normal, the Shapiro-Wilk test was performed. The terms range (minimum and maximum), mean, standard deviation, median, and interquartile range were

used to characterize quantitative data. The 5% level was used to assess the results' significance. The tests that were employed included the receiver operating characteristic curve (ROC), Student t test, Mann-Whitney U test, Fisher exact or Monte Carlo correction, and chi-square test.

Results

Our results on the demographic data cleared that, in studied cases group the male was more susceptible to HIE than the female neonates. Also, the gestational age among studied cases was lower than that of the normal healthy baby. While, the body weight of the studied cases was lower than the body weight of normal healthy baby (Table 1).

While our results on the obstetric characters cleared that, the maternal age of studied patients was less than that of the normal healthy baby and the parity number in patient cases was 8 (26.70%) and 22 (73.30%) for primiparous and multiparous, respectively with a mean value of 2.67 $\pm$ 1.45 while, in control group were 6 (20 %) and 24 (80 %) for primiparous and multiparous, respectively with a mean value of 2.47 $\pm$ 1.14. while, the number of babies was 1.63 $\pm$ 1.35 and 2.03 $\pm$ 1.0 for patient and control group, respectively (Table 1).

The mode of delivery in studied cases was 15 (50%) and 15 (50%) for cesarean section (CS) and vaginal delivery in patient group and in control group was 21 (70%) CS and 9 (30%) vaginal delivery. There was obstructed labor in 21 (70%) of cases while the labor was not obstructed in 100% of controls (Table 1).

Regarding the conscious level, 100% of control neonates were conscious, while, in studied cases the conscious percent was 66.70 % and those who were no conscious were 33.30%. While, the muscle tone of normal level in studied cases was 43.3% and in controls was 100 %. The atonia appears only in studied cases (16.70%) and also hypotonia appears only in studied cases (40%). The moro reflex in studied cases was 33.3 % and in control group was 100%. The respiratory distress appears only in 26.70 % of studied cases (Table 2).

The CBC (hemoglobin [Hb], white blood cells [WBCs], platelets) levels differ significantly ($P < 0.01$) among the patient and control group. The Hb level in examined cases showed a lower level than its level in control group (12.95 $\pm$ 2.39 vs. 14.19 $\pm$ 0.73). The WBCs ($\times 10^3/\mu\text{L}$) level in examined cases showed a higher level than its level in control group (16.43 $\pm$ 6.32 vs. 13.31 $\pm$ 1.74). The platelets ($\times 10^3/\mu\text{L}$) level in examined cases showed a lower level than its level in control group (141.8 $\pm$ 93.27 vs. 297.7 $\pm$ 75.0) (Table 3).

The serum sodium level was significantly lower among the cases than controls ($P < 0.001$) (131.5 $\pm$ 6.78 vs. 138.7 $\pm$ 4.08).

Table 1. Patients, demographic and obstetric data by study group

Variable	Cases (n=30)	Control (n=30)	Test of Sig.	P value
Sex, n (%)			$\chi^2=3.300$	0.069
Male	20 (66.7)	13 (43.3)		
Female	10 (33.3)	17 (56.7)		
Gestational age (wk)			U=270.0	0.005
Range	33.0–40.0	37.0–40.0		
Mean±SD	36.83±1.72	37.97±1.0		
Median (IQR)	37.0 (37.0–38.0)	38.0 (37.0–38.0)		
Maternal age (yr)			t=1.282	0.205
Range	19.0–45.0	22.0–40.0		
Mean±SD	30.40±6.98	32.50±5.64		
Median (IQR)	32.0 (25.0–35.0)	35.50 (27.0–36.0)		
Parity				
Primi, n (%)	8 (26.7)	6 (20.0)	$\chi^2=0.373$	0.542
Multi, n (%)	22 (73.3)	24 (80.0)	U=422.0	0.670
Range	1.0–7.0	1.0–5.0		
Mean±SD	2.67±1.45	2.47±1.14		
Median (IQR)	2.50 (1.0–4.0)	2.0 (2.0–3.0)		
No. of babies			U=364.50	0.192
Range	0.0–5.0	1.0–4.0		
Mean±SD	1.63±1.35	2.03±1.0		
Median (IQR)	1.50 (0.0–3.0)	2.0 (1.0–3.0)		
Mode of delivery, n (%)			$\chi^2=2.500$	0.114
CS	15 (50.0)	21 (70.0)		
Vaginal delivery	15 (50.0)	9 (30.0)		
Obstructed labor, n (%)			$\chi^2=32.308$	<0.001
Yes	21 (70.0)	0 (0)		
No	9 (30.0)	30 (100)		

SD, standard deviation; IQR, interquartile ranges; CS, cesarean section; t, Student t test; U, Mann-Whitney U test; χ^2 , chi-square test. Boldface indicates a statistically significant difference with $P<0.05$.

Table 2. Patients, neurological examination results by study group

Variable	Cases (n=30)	Control (n=30)	χ^2	P value
Conscious			12.0	0.001
No	10 (33.3)	0 (0)		
Yes	20 (66.7)	30 (100)		
Muscle tone				
Normal	13 (43.3)	30 (100)	25.314	<0.001^{a)}
Atonia	5 (16.7)	0 (0)		
Hypotonia	12 (40)	0 (0)		
Moro reflex	10 (33.3)	30 (100)	30.0	<0.001
Convulsion	24 (80)	0 (0)	40.0	<0.001
Respiratory distress	8 (26.7)	0 (0)	9.231	0.005^{b)}

Values are presented as number (%).

^{a)}Monte Carlo test. ^{b)}Fisher exact test.

Boldface indicates a statistically significant difference with $P<0.05$.

The level of serum calcium level was significantly lower among the cases than controls ($P<0.001$) (8.48 ± 0.65 vs. 9.58 ± 0.47) (Table 3).

The CRP level was significantly higher among the cases than controls ($P=0.001$), (6.41 ± 1.88 vs. 4.80 ± 1.49). The urea level was significantly higher among the cases than controls differ ($P<0.001$) (59.83 ± 28.92 vs. 24.13 ± 8.17). The crea-

tinine level also, was significantly higher among the cases than controls ($P<0.001$) (1.71 ± 0.88 vs. 1.16 ± 0.26) (Table 3).

The alanine aminotransferase (ALT) level was significantly higher among the patient group than control group ($P<0.001$) (66.33 ± 34.84 vs. 30.27 ± 4.95). The aspartate aminotransferase (AST) level differs significantly among the patient and control group ($P<0.001$), where its level in patient group was 52.97 ± 31.46 and in control group was 20.87 ± 2.96 (Table 3).

The arterial blood gases shown marked metabolic acidosis reached to be statically significant with PH 7.1 ± 0.05 in cases versus control 7.37 ± 0.03 ($P<0.001$), also mean HCO_3 level was 12.01 ± 2.60 in cases while 21.23 ± 2.18 in control with ($P<0.001$) (Table 3).

The miRNA-410 level differs significantly among the patient and control group ($P=0.027$), where its level in patient group was 1.56 ± 6.43 and in control group was 0.58 ± 0.60 .

The miRNA-498 level was significantly higher among the studied cases than controls ($P=0.015$), (55.63 ± 118.24 vs. 3.74 ± 7.09) (Table 4).

The ROC curve for miRNA-410 cleared that, the area under the curve (AUC) was 0.666 with sensitivity of 70%

Table 3. Laboratory test results by study group

Laboratory test	Cases (n=30)	Control (n=30)	Test of Sig.	P value
Hb (g/dL)			t=2.700	0.011
Range	8.60–17.40	12.70–15.20		
Mean±SD	12.95±2.39	14.19±0.73		
Median (IQR)	12.80 (10.80–14.80)	14.30 (13.80–14.80)		
WBCs (×10 ³ /μL)			U=299.50	0.026
Range	6.0–31.50	10.0–17.0		
Mean±SD	16.43±6.32	13.31±1.74		
Median (IQR)	15.50 (12.30–17.70)	13.0 (12.0–13.80)		
Platelets (×10 ³ /μL)			U=98.0	<0.001
Range	35.0–360.0	190.0–450.0		
Mean±SD	141.8±93.27	297.7±75.0		
Median (IQR)	107.5 (65.0–227.0)	285.0 (240.0–350.0)		
Serum sodium (Na ⁺) (mEq/L)			5.033	<0.001
Range	122.0–150.0	132.0–145.0		
Mean±SD	131.5±6.78	138.7±4.08		
Median (IQR)	130.0 (125.0–137.0)	139.5 (135.0–142.0)		
Serum potassium (K ⁺) (mEq/L)			1.375	0.177
Range	3.0–7.20	3.40–5.0		
Mean±SD	4.38±1.13	4.07±0.48		
Median (IQR)	4.25 (3.50–4.80)	4.10 (3.70–4.30)		
Ca (mg/dL)			7.532	<0.001
Range	7.30–10.0	9.0–10.50		
Mean±SD	8.48±0.65	9.58±0.47		
Median (IQR)	8.35 (8.0–9.0)	9.50 (9.0–10.0)		
CRP (mg/L)			3.684	0.001
Range	3.0–9.0	3.0–6.0		
Mean±SD	6.41±1.88	4.80±1.49		
Median (IQR)	6.0 (6.0–8.80)	6.0 (3.0–6.0)		
Urea (mg/dL)			t=6.506	<0.001
Range	10.0–120.0	12.0–40.0		
Mean±SD	59.83±28.92	24.13±8.17		
Median (IQR)	53.0 (38.0–85.0)	25.0 (18.0–30.0)		
Creatinine(mg/dL)			U=191.50	<0.001
Range	0.90–5.70	0.50–1.60		
Mean±SD	1.71±0.88	1.16±0.26		
Median (IQR)	1.65 (1.20–1.90)	1.20 (0.90–1.40)		
Liver enzymes				
ALT (U/L)			147.50	<0.001
Range	10.0–125.0	20.0–39.0		
Mean±SD	66.33±34.84	30.27±4.95		
Median (IQR)	60.0 (35.0–102.0)	29.0 (28.0–35.0)		
AST (U/L)			40.500	<0.001
Range	18.0–142.0	16.0–29.0		
Mean±SD	52.97±31.46	20.87±2.96		
Median (IQR)	48.0 (29.0–71.0)	21.0 (19.0–22.0)		
Arterial blood gas				
PH, mean±SD	7.1±0.05	7.37±0.03		<0.001
PCO ₂ , mean±SD	27.3±4.57	37.4±3.80		<0.001
PO ₂ , mean±SD	60.5±7.38	66.2±2.09		0.008
HCO ₃ (mEq/L), mean±SD	12.01±2.60	21.23±2.18		<0.001

SD, standard deviation; IQR, interquartile ranges; Hb, hemoglobin; WBC, white blood cell; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; U, Mann-Whitney *U* test; t, Student *t* test.

Boldface indicates a statistically significant difference with *P*<0.05.

and specificity was 60%, the cutoff point ≤ 0.242 , while the positive predictive value (PPV) was 63.60 and negative predictive value (NPV) was 66.70. Also, the results of the ROC curve for miRNA-498 cleared that, the AUC was 0.683 with sensitivity of 70% and specificity was 66.67%, the cutoff point >1.854 , while the PPV was 67.70 and NPV was 69.00 (Figs. 1 and 2; Table 5).

The miRNA-498 was significantly positive correlated with CRP in cases (Table 6).

Discussion

Neonatal HIE causes long-term neurologic morbidity and is linked to significant newborn mortality.¹⁶⁾ The only known therapy for neonatal HIE at present time is therapeutic hypothermia, however it must be administered within 6 hours of delivery and is not recommended in situations of severe HIE.¹⁷⁾ Finding new biomarkers for diagnosis in this population might help forecast brain damage, distinguish newborns with HIE from healthy babies or those who have low-cord pH, and support doctors in making decisions in the moment.²⁾

Our results showed that the obstructed labor was found in 70% (21 patients) of cases versus 0% in control groups.

These results agreed with those of Johnston et al.¹⁸⁾ who stated that asphyxia leading to brain ischemia and hypoxia are the fundamental physiological mechanisms that cause HIE in both preterm and term neonates. A chain of reactions, that includes acidosis, the release of inflammatory mediators, and the creation of free radicals, are brought on by hypoperfusion combined with hypoxia. These biological compounds cause widespread brain dam-

age and lack of normal cerebral autoregulation. The degree of brain maturation, length, and severity of hypoxia all affect the precise kind of damage. Myelinated fibers are more metabolically active in term newborns, making them more susceptible to HIE.

Our results on the maternal diseases and maternal drugs cleared that, the most important maternal diseases include preeclampsia, followed by diabetes mellitus and polyhydramnios and the mothers that take drugs were 11 (36.70%) and those not take drugs were 19 (63.30%).

The Apgar level at 5 and 9 minutes in examined cases was lower than its level in control healthy baby. These results attributed to the abnormal electroencephalographic readings that may indicate a poor prognosis, such as long-term neurological consequences or approaching mortality.

The results^{19,20)} of were in agreement with our findings, which indicated that low Apgar scores (bradycardia, inadequate respiratory effort, hypotonia, reduced alertness,

Table 6. Correlation between miRNA-410 and miRNA-498 and different parameters in cases group (n=30)

Variable	miRNA-410		miRNA-498	
	<i>r_s</i>	<i>P</i> value	<i>r_s</i>	<i>P</i> value
Gestational age (wk)	-0.176	0.353	-0.185	0.328
Weight (kg)	-0.007	0.971	0.027	0.886
Maternal age (yr)	-0.206	0.274	0.075	0.694
Parity	-0.074	0.697	0.028	0.883
No. of babies	-0.074	0.697	0.028	0.883
Apgar 5 min	0.081	0.670	-0.401	0.028
Apgar 10 min	-0.091	0.632	-0.413	0.023
Hb (g/dL)	-0.269	0.151	-0.136	0.474
WBCs ($\times 10^3/\mu\text{L}$)	-0.007	0.971	-0.166	0.379
Platelets ($\times 10^3/\mu\text{L}$)	-0.163	0.388	-0.133	0.482
Serum sodium (Na^+)	-0.047	0.805	-0.133	0.484
Serum potassium (K^+)	-0.087	0.647	-0.232	0.218
Ca (mg/dL)	-0.075	0.695	-0.016	0.932
CRP (mg/L)	0.247	0.189	0.447	0.013
Urea (mg/dL)	0.012	0.950	0.142	0.453
Creatinine (mg/dL)	-0.196	0.300	0.097	0.611
ALT (U/L)	0.043	0.821	-0.063	0.742
AST (U/L)	0.040	0.835	0.001	0.996
pH	-0.104	0.583	-0.170	0.369
PaCO ₂	0.102	0.592	0.025	0.894
PaO ₂	-0.012	0.948	0.059	0.756
HCO ₃	0.034	0.859	0.059	0.757

Hb, hemoglobin; WBC, white blood cell; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; *r_s*, Spearman coefficient. Boldface indicates a statistically significant difference with $P < 0.05$.

Table 4. Levels of miRNA-410 and miRNA-498 by study groups

Variable	Cases (n=30)	Control (n=30)	<i>U</i>	<i>P</i> value
miRNA-410			300.50	0.027
Range	0.001–35.42	0.002–2.25		
Mean $\pm$ SD	1.56 $\pm$ 6.43	0.58 $\pm$ 0.60		
Median (IQR)	0.04 (0.01–0.52)	0.46 (0.09–1.0)		
miRNA-498			285.0	0.015
Range	0.002–563.16	0.003–28.47		
Mean $\pm$ SD	55.63 $\pm$ 118.24	3.74 $\pm$ 7.09		
Median (IQR)	8.93 (0.28–53.45)	1.07 (0.42–2.31)		

SD, standard deviation; IQR, interquartile range; *U*, Mann-Whitney *U* test. Boldface indicates a statistically significant difference with $P < 0.05$.

Table 5. Ability of miRNA-410 and miRNA-498 expression to discriminate between cases (n=30) and controls (n=30)

	AUC	<i>P</i> value	95% CI	Cutoff	Sensitivity	Specificity	PPV	NPV
miRNA-410	0.666	0.027	0.523–0.809	≤ 0.242	70.0	60.00	63.6	66.7
miRNA-498	0.683	0.015	0.538–0.829	>1.854	70.0	66.67	67.7	69.0

AUC, area under the curve; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value. Boldface indicates a statistically significant difference with $P < 0.05$.

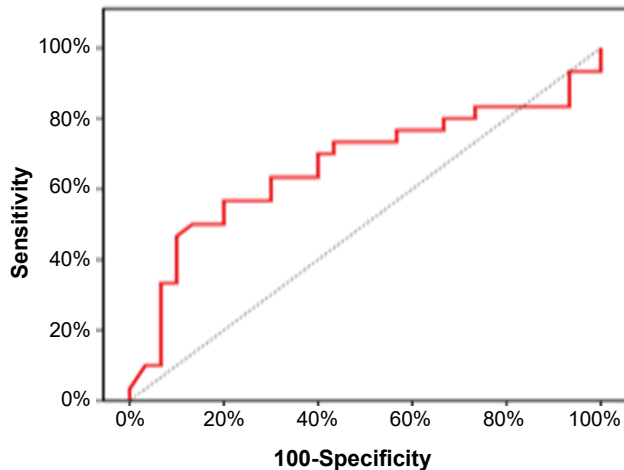


Fig. 1. Receiver operating characteristic curve of ability of miRNA-410 to discriminate between patients (n=30) and controls (n=30).

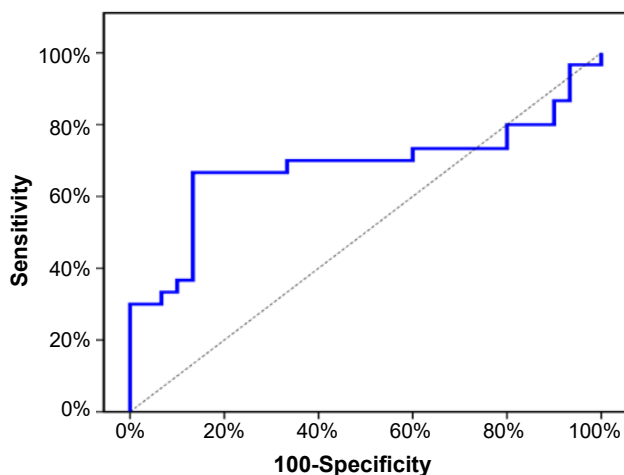


Fig. 2. Receiver operating characteristic curve of ability of miRNA-498 to discriminate between patients (n=30) and controls (n=30).

weak or absent cry, aberrant skin color) and metabolic acidosis in cord blood were possible in HIE neonates. Seizures often start to occur in the first 24 hours of life for the baby. Spastic cerebral palsy in children with periventricular leukomalacia can manifest as diplegia, quadriplegia, or hemiplegia. Subcortical white matter involvement results in profound mental disability and visual impairment. Extrapyramidal symptoms are caused by involvement of the thalamus and basal ganglia. Quadriplegia, bulbar and choreoathetoid symptoms, microcephaly, and mental retardation are all linked to multicystic encephalopathy.

While, our results on the different studied parameters cleared that, the conscious level, muscle tone, moro reflex, were lower in neonates suffering from neonatal HIE than the control group and the atonia, hypotonia, Res. Distress appear only in studied cases.

These results agreed with the results of⁽²¹⁾ where they reported that, the main symptoms of neonatal HIE include

seizures, coma and hypotonia. The results of CBC cleared that, the Hb and platelets levels decreased in neonatal HIE than the control group while, the WBCs level in examined cases showed a higher level than its level in control group.

These results agreed with the results of Lelubre et al.⁽²²⁾ who found that severe Hb deficiency in neonatal HIE causes blood flow of the brain and oxygen saturation of the arteries to directly correlate with brain oxygen delivery. They also found that a significant decrease in Hb may result in reduced oxygen supply to the brain and ultimately hypoxia of the tissues if compensating systems intended to maintain sustained oxygenation of the tissue fail or become overwhelmed. prenatal anemia is one of the factors that contribute to mild to severe prenatal asphyxia. Also, Kalteren et al.⁽²³⁾ showed that in severely anemic asphyxiated newborns, a lower initial Hb level was a significant predictor of suboptimal neurodevelopmental outcome.

It has been demonstrated that the duration of fetal bradycardia affects the rising level of WBCs linked to lymphocyte levels in neonates. The number of lymphocytes increased when fetal bradycardia lasted more than 25 minutes, but these values were only temporary since they quickly returned to normal between 18 and 24 hours, which limited their applicability as a diagnostic for asphyxia. This fluctuation in the number of lymphocytes has been linked to a stress reaction that might start in the thymus.⁽²⁴⁾

Our results on the electrolytes cleared that, the serum sodium and calcium levels decreased in HIE than the control group and the potassium level increased than the control group.⁽²⁵⁾

The CRP, urea and creatinine levels increased in neonatal HIE than that of control healthy baby. Also, the random blood glucose level increased in studied cases than the control healthy baby.

These results agreed with the results of Alsulaimani et al.⁽²⁶⁾ who stated that brain inflammatory cytokines are more quickly expressed when cerebral hypoxia-ischemia occurs, and this triggers a reaction of inflammatory cells. According to research, HIE was not always associated with a rise in markers of inflammation, including CRP,⁽²⁷⁾ which is consistent with our study's findings that only 15.8% of patients had high CRP levels.

Also, the ALT and AST increased in studied cases than the control healthy groups. The increasing level of AST and ALT attributed to the injury of the liver resulted from hypoxia resulted from neonatal HIE.

These results agreed with the results of Choudhary et al.⁽²⁸⁾ who revealed that, any system in the body can be harmed by hypoxia. When the fetus is insulted by hypoxic-ischemic conditions, a sequence of reflexes and actions take place to safeguard vital organs (brain, adrenal glands,

heart) and adjust cardiac output at expense of others (kidneys, liver, gastrointestinal system, lungs, spleen).

Recent researches have indicated that miRNAs have important functions in the emergence and advancement of disorders connected to brain damage.²⁹⁾

The miRNA-410 results cleared that, its level in cases was greater than the controls where its level in cases was 1.56 ± 6.43 and in control group was 0.58 ± 0.60 .

These findings were in line with those of O'Sullivan, who examined the alterations of miRNAs expressions in cord blood in newborns suffering from HIE. Among the deregulated miRNAs in whole blood samples is micro RNA-410 (miR-410).¹¹⁾ Furthermore, it has been shown that miR-410 guards against brain damage in Parkinson disease,¹²⁾ anesthesia-induced cognitive dysfunction¹³⁾ and ischemic stroke.³⁾

Also, our results are in line with the findings of,^{12,13)} who showed that HIE neonates' blood levels of miR-410 were downregulated in comparison to healthy controls. Furthermore, the expression of miR-410 between babies with varying degrees of HIE was much different. Therefore, we hypothesized that miR-410 may have a part in the onset and development of newborn HIE.

Low levels of miR-498 expression were seen in ovarian and non-small cell lung cancer.³⁰⁾

Our results on the 14-miRNA-498 among the patient and control group, where its level in patient group was 55.63 ± 118.24 and in control group was 3.74 ± 7.09 .

These results aligned with the findings of Matamala et al.³¹⁾ who found miR-498 overexpression in triple negative breast cancer (TNBC) cell lines based on qRT-PCR studies and documented upregulation of miR-498 in TNBC tissues based on microarray results. Furthermore, compared to primary medullary thyroid cancer, miR-498 is substantially greater in metastatic medullary thyroid carcinoma.³²⁾

Additionally, our findings corroborated those of Kasiapap et al.,³³⁾ who reported the variability of miR-498 expression patterns across several organs. Furthermore, reports have indicated that miR-498 hits other targets besides PTEN. For example, in an estrogen receptor- α -dependent way, telomerase reverse transcriptase promotes cell proliferation. Vitamin D-induced miR-498 overexpression, which targets telomerase reverse transcriptase mRNA, functions as a tumor suppressor and decreases telomerase reverse transcriptase levels in ovarian cancer cells.

Hypoxic stress regulates the expression of an expanding but specific subset of miRNAs, termed hypoxamirs. Although the specific miRNA hypoxic signature can vary based on the cellular or physiological scenario, a core group of hypoxamirs appears to be modulated consistently by hypoxia in diverse contexts, one of them is microRNA498.

Among these, multiple hypoxamirs directly target important gene transcripts that coordinate metabolic reprogramming, DNA repair, apoptosis, and angiogenesis, among many other cellular adaptations to low oxygen availability.³⁴⁾

The outcome of the children cleared that, discharged babies were 20 (66.70%) and the died babies were 10 (33.30%). High newborn mortality and long-term neurologic morbidity are linked to ischemic brain damages mostly induced by perinatal hypoxia, which is also responsible for the high mortality from HIE.¹⁶⁾

The ROC curve for miRNA-410 to discriminate patients cleared that, the AUC was 0.666 with sensitivity of 70% and specificity was 60%, the cutoff point ≤ 0.242 , while the PPV was 63.60 and NPV was 66.70. Also, the results of the ROC curve for miRNA-498 to discriminate patients cleared that, the AUC was 0.683 with sensitivity of 70% and specificity was 66.67%, the cutoff point > 1.854 , while the PPV was 67.70 and NPV was 69.00. These results indicated that miRNA-410 and miRNA-498 can be used as an auxiliary diagnostic and prognostic tool in HIE.

In 2024, Ichiro Wakabayashi et al.³⁵⁾ found that, there were significant differences in serum and plasma levels of about one-third of the miRNAs ($n=299$) at time 0. Serum levels of 249 miRNAs were significantly higher than their plasma levels, while plasma levels of 50 miRNAs were significantly higher than their serum levels. Some of these differences were speculated to be due to clot formation during preparation of serum since miRNA-containing blood cells are included in the clot

In conclusion, the male was more susceptible to neonatal HIE than the female baby. MiRNA-410 and miRNA-498 can be used as an auxiliary tool in the diagnosis and prognosis of HIE.

Footnotes

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References

1. Laptook AR. Birth asphyxia and hypoxic-ischemic brain injury in the preterm infant. *Clin Perinatol* 2016;43:529-45.
2. Dakroub F, Kobeissy F, Mondello S, Yang Z, Xu H, Sura L, et al. MicroRNAs as biomarkers of brain injury in neonatal encephalopathy: an observational cohort study. *Sci Rep* 2024;14:6645.
3. Liu G, Li ZG, Gao JS. Hypothermia in neonatal hypoxic-ischemic encephalopathy (HIE). *Eur Rev Med Pharmacol Sci* 2017;21(4 Suppl):50-3.
4. Montaldo P, Burgod C, Herberg JA, Kaforou M, Cunningham AJ, Mejias A, et al. Whole-blood gene expression profile after hypoxic-ischemic encephalopathy. *JAMA Netw Open* 2024;7:e2354433.
5. Chao LM, Sun W, Chen H, Liu BY, Li PF, Zhao DW. Micro RNA-31 inhibits osteosarcoma cell proliferation, migration and invasion by targeting PIK3C2A. *Eur Rev Med Pharmacol Sci* 2018;22:7205-13.
6. Vishnoi A, Rani S. MiRNA biogenesis and regulation of diseases: an overview. *Methods Mol Biol* 2017;1509:1-10.
7. Bertoli G, Cava C, Castiglioni I. MicroRNAs: new biomarkers for diagnosis, prognosis, therapy prediction and therapeutic tools for breast cancer. *Theranostics* 2015;5:1122-43.
8. Ma Q, Dasgupta C, Li Y, Bajwa NM, Xiong F, Harding B, et al. Inhibition of microRNA-210 provides neuroprotection in hypoxic-ischemic brain injury in neonatal rats. *Neurobiol Dis* 2016;89:202-12.
9. Chen D, Dixon BJ, Doycheva DM, Li B, Zhang Y, Hu Q, et al. Nowrangi D, Flores J, Filippov V, Zhang JH, Tang J. IRE1 α inhibition decreased TXNIP/NLRP3 inflammasome activation through miR-17-5p after neonatal hypoxic-ischemic brain injury in rats. *J Neuroinflammation* 2018;15:32.
10. Chen R, Wang M, Fu S, Cao F, Duan P, Lu J. MicroRNA-204 may participate in the pathogenesis of hypoxic-ischemic encephalopathy through targeting KLLN. *Exp Ther Med* 2019;18:3299-306.
11. O'Sullivan MP, Looney AM, Moloney GM, FINDER M, Hallberg B, Clarke G, et al. Validation of altered umbilical cord blood MicroRNA expression in neonatal hypoxic-ischemic encephalopathy. *JAMA Neurol* 2019;76:333-41.
12. Ge H, Yan Z, Zhu H, Zhao H. MiR-410 exerts neuroprotective effects in a cellular model of Parkinson's disease induced by 6-hydroxydopamine via inhibiting the PTEN/AKT/mTOR signaling pathway. *Exp Mol Pathol* 2019;109:16-24.
13. Su R, Sun P, Zhang D, Xiao W, Feng C, et al. Neuroprotective effect of miR-410-3p against sevoflurane anesthesia-induced cognitive dysfunction in rats through PI3K/Akt signaling pathway via targeting C-X-C motif chemokine receptor 5. *Genes Genomics* 2019;41:1223-31.
14. Meng Q, Yang P, Lu Y. MicroRNA-410 serves as a candidate biomarker in hypoxic-ischemic encephalopathy newborns and provides neuroprotection in oxygen-glucose deprivation-injured PC12 and SH-SY5Y cells. *Brain Behav* 2021;11:e2293.
15. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976;33:696-705.
16. Glass HC. Hypoxic-ischemic encephalopathy and other neonatal encephalopathies. *Continuum (Minneapolis)* 2018;24(1, Child Neurology):57-71.
17. Chiang MC, Jong YJ, Lin CH. Therapeutic hypothermia for neonates with hypoxic ischemic encephalopathy. *Pediatr Neonatol* 2017;58:475-83.
18. Johnston MV, Trescher WH, Ishida A, Nakajima W. Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatr Res* 2001;49:735-41.
19. Shalak L, Perlman JM. Hypoxic-ischemic brain injury in the term infant-current concepts. *Early Hum Dev* 2004;80:125-41.
20. Elsadek AE, FathyBarseem N, Suliman HA, Elshorbagy HH, Kamal NM, Talaat IM, et al. Hepatic injury in neonates with perinatal asphyxia. *Glob Pediatr Health* 2021;8:2333794 X20987781.
21. Eghbalian F. Frequency of hypoxic-ischemic encephalopathy among hospitalized neonates in West Iran. *Iran J Pediatr* 2010;20:244-5.
22. Lelubre C, Bouzat P, Crippa IA, Taccone FS. Anemia management after acute brain injury. *Crit Care* 2016;20:152.
23. Kalteren WS, Ter Horst HJ, den Heijer AE, de Vetten L, Kooi EM, Bos AF. Perinatal anemia is associated with neonatal and neurodevelopmental outcomes in infants with moderate to severe perinatal asphyxia. *Neonatology* 2018;114:315-22.
24. Dina P, Muraskas JK. Hematologic changes in newborns with neonatal encephalopathy. *Neoreviews* 2018;19:e29-33.
25. Manjunatha Babu R, Jerry PK, Harish G, Susheela C. A comparative study of serum electrolytes in newborns with birth asphyxia and non-asphyxiated newborns. *Int J Pediatr Res* 2018;5:407-12.
26. Alsulaimani AA, Abuelsaad AS, Mohamed NM. Inflammatory cytokines in neonatal hypoxic ischemic encephalopathy and their correlation with brain marker S100 protein: a case control study in Saudi Arabia. *J Clin Cell Immunol* 2015;6:1000289.
27. Saito J, Shibasaki J, Shimokaze T, Kishigami M, Ohyama M, Hoshino R, et al. Temporal relationship between serum levels of interleukin-6 and C-reactive protein in therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *Am J Perinatol* 2016;33:1401-6.
28. Choudhary M, Sharma D, Dabi D, Lamba M, Pandita A, Shastri S. Hepatic dysfunction in asphyxiated neonates: prospective case-controlled study. *Clin Med Insights Pediatr* 2015;9:1-6.
29. Sarkar SN, Russell AE, Engler-Chiurazzi EB, Porter KN, Simpkins JW. MicroRNAs and the genetic nexus of brain aging, neuroinflammation, neurodegeneration, and brain trauma. *Aging Dis* 2019;10:329-52.
30. Wang M, Zhang Q, Wang J, Zhai Y. MicroRNA-498 is downregulated in non-small cell lung cancer and correlates with tumor progression. *J Cancer Res Ther* 2015;11 Suppl 1:C107-11.

31. Matamala N, Vargas MT, González-Cámpora R, Arias JI, Menéndez P, Andrés-León E, et al. MicroRNA deregulation in triple negative breast cancer reveals a role of miR-498 in regulating BRCA1 expression. *Oncotarget* 2016;7:20068-79.
32. Santarpia L, Calin GA, Adam L, Ye L, Fusco A, Giunti S, et al. A miRNA signature associated with human metastatic medullary thyroid carcinoma. *Endocr Relat Cancer* 2013;20: 809-23.
33. Kasiappan R, Shen Z, Tse AK, Jinwal U, Tang J, Lungchukiet P, et al. 1,25-Dihydroxyvitamin D3 suppresses telomerase expression and human cancer growth through micro RNA-498. *J Biol Chem* 2012;287:41297-309.
34. Pocock R. Invited review: decoding the microRNA response to hypoxia. *Pflugers Arch* 2011;461:307-15.
35. Wakabayashi I, Marumo M, Ekawa K, Daimon T. Differences in serum and plasma levels of microRNAs and their time-course changes after blood collection. *Pract Lab Med* 2024;39:e00376.

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