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ORIGINAL ARTICLE

Diagnostic Utility of Aquaporin-4 Blood Level in Neonatal Hypoxic Ischemic Encephalopathy

Ehab A. Al-Banna[1], Noha A. Rezk[2], Ahmed M. Abdel Moniem[1], Sabah H. Abdel Rahman[1*]

[1]Pediatrics Department, Faculty of Medicine, Zagazig University

[2] Medical Biochemistry Department, Faculty of Medicine, Zagazig University

***Corresponding author:**
Sabah H. Abdel Rahman,
Pediatrics Department,
Faculty of
Medicine,Zagazig
University.
Email: Sabah.hassan@yahoo.com

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ABSTRACT

Background: In neonates, cerebral hypoxic-ischemia is the main cause of brain oedema which results in neurodevelopmental dysfunction. After cerebral injury, Aquaporin-4 (AQP4) is reported to be overexpressed in the astrocytes. This study was conducted to evaluate using of the blood level of aquaporin-4 in diagnosis of hypoxic ischemic encephalopathy in neonates.

Methods: Blood samples were collected from 30 neonates with hypoxic ischemic encephalopathy (HIE), sample (1) at age of 2 days while sample (2) at age of 7 days, and from 30 neonates control groups. The collected blood samples were used to measure aquaporin-4 level and creatine phosphokinase (CPK). The study was conducted, during a period of 10 months, at Neonatal Intensive Care Unit, Pediatric Department, General Beni-Suef Hospital, and this study was case control study.

Results: Aquaporin-4 at age of 2 days was found to be significantly higher in hypoxic patients than the control (220.83±98.85 vs. 135.51± 28.32 pg/ml). Moreover, the level of AQP4 at age of 7 day was 349.72±110.25 pg/ml. There was no significant difference in AQP4 level between preterm and full term hypoxic patients at age of 2 days, as the values were 275.33±69.72 pg/ml for preterm and 214.55±97.06 for full term. However, there was a significant difference in AQP4 level between preterm and full term hypoxic patients at age of 7 days, as the values were 453.93±66.19 pg/ml for preterm and 339.93± 108.22 pg/ml for full term. At age of 2 and 7 days, the severe hypoxic patients had significantly higher AQP4 levels than moderate or mild hypoxic patients.

Conclusions: The obtained results indicate that serum Aquaporin-4 level and CPK enzyme activity could be used as early predictors in diagnosis of HIE.

Keywords: Aquaporin-4; Creatine phosphokinase; Ischemic encephalopathy; Neonates

INTRODUCTION

Aquaporin-4 (AQP4) is a portion of Aquaporins family, it is profuse in the brain and spinal cord, where it is highly polarized, with concentrated expression in astrocyte end-feet which envelop capillaries [1]. After cerebral injury, Aquaporin-4 is reported to be overexpressed in the astrocytes. AQP4 organizes water changes inside and out of the brain parenchyma [2].

In neonates, cerebral hypoxic-ischemia is the main cause of brain oedema which results in neurodevelopmental dysfunction [3]. Also, neonatal hypoxic ischemic encephalopathy (HIE) can be distinguished as an injury which occurs in the immature brain, leading to delayed cell death through inflammation, excitotoxicity, and oxidative stress [4]. The pathophysiology including neonatal HIE consists of several phases. The first phase is a decrease in systematic arterial blood pressure and changes in the vasculature, resulting in high risk for ischemic brain injury and tissue acidosis [5]. The following next phase is a primary energy damage phase that takes place at the cellular stage so cellular energy metabolism alters to a dependence on anaerobic metabolism that results in lactic acid collection and adenosine triphosphate (ATP) reduction [6]. Also, loss of cellular homeostasis causes accumulation of water, calcium, sodium in the cells, and excitatory neurotransmitter release leading to an “excitotoxic-

oxidative cascade". Excessive neurotransmitter receptors stimulation and membrane depolarization leads to increasing cellular calcium influx [7]. Moreover, further calcium influx results in increased lipase activation, causing fatty acids release and increased activity of neuronal nitric oxide synthase, leading to production of free radicals and mitochondrial dysfunction. Consequently, mitochondrial dysfunction ultimately indicates pathways of apoptotic or necrotic cell death. Apoptotic cell death occurs when energy supplies from cultured astrocytes in an aquaporin-4-dependent manner [8], which may influence the pathological results, as well. These data indicate that aquaporin-4 is an inflammatory mediator and has a differential role during oedema build-up and resolution [9]. Aquaporin-4 has been found to contribute in the swelling of astrocytes during formation of the brain oedema, while in the resolution phase it has been shown to increase the reabsorption of extracellular fluid [10]. The present study was conducted to evaluate using of the blood level of aquaporin-4 in diagnosis of hypoxic ischemic encephalopathy in neonates, and also to investigate the relationship between the aquaporin-4 blood level and the severity of hypoxia.

METHODS

The study was performed at Neonatal Intensive Care Unit, Pediatric Department, General Beni-Suef Hospital, and at Pediatrics and Medical Biochemistry Departments, Faculty of Medicine, Zagazig University.

The blood samples were collected from thirty hypoxic neonates, sample (1) at age of 2 days while sample (2) at age of 7 days and also thirty samples from healthy control neonates. The collected blood samples were used to measure the aquaporin-4 level and creatine phosphokinase. The study was conducted during a period of 10 months, and this study was case control study.

The cases were 30 neonates, both inborn and out born, admitted to the Neonatal Intensive Care Unit at Pediatric Department, General Beni-Suef Hospital, diagnosed as hypoxic ischemic encephalopathy according to the American Academy of pediatrics (AAP) and the American College of Obstetricians and Gynecologists (ACOG) [11] Asphyxia was defined according to an Apgar score less than 3 at the 5th min, pH less than 7.0, or base excess less than 12 in cord blood or venous blood taken from newborns within 60 min of birth, or the need for positive pressure ventilation (more than 3 min) [12]. Infants who fulfilled three or more of the above clinical and biochemical criteria were developed HIE, as defined by Levene staging [13]. In this study, thirteen infants of 30 neonates were delivered by emergency cesarean section and seventeen were delivered vaginally.

The controls were 30 healthy neonates at the same gestational age delivered by elective cesarean section (n =13) or vaginally (n=17) , fulfilling the criteria of no maternal illness, no signs of fetal distress, pH >7.2 in cord blood or venous blood, and Apgar scores at 1 and 5 min >7.

Neonates with any malformation, systemic infection, intrauterine growth retardation, or cardiac or hemolytic disease were excluded from the study. Other exclusion criteria were multiple pregnancies, history of neurological disease in the family, any consanguineous marriage, congenital or perinatal infections including chorioamnionitis, and maternal drug addiction, hypertension, or diabetes. All the studied neonates were subjected to complete antenatal and natal history was taken, assessment of Apgar score at 1and 5 minutes was done by neonatal resuscitation program (NRP) guidelines [14,15], assessment of gestational age using Ballard scoring system [16], birth weight was measured, and clinical neurological examination was done to detect patients with HIE, and when present its stage was assessed according to Sarnat and Sarnat scoring system [17]. Moreover, a number of anti-convulsant drugs, where no, one and two anti-convulsants, was used in controlling of seizures.

The blood samples (2 ml) were collected twice in the hypoxic neonates and controls groups, one at two days of age and the second at seven days. These blood samples were collected in plastic tubes, and then centrifuged at 3000 r.p.m for 15 minutes. Then, the serum was separated in epiendurf tubes and stored at – 20 °C until the chemical analyses by Human Aquaporin 4(AQP-4) kit. The Kit uses adouble-antibody sandawich enzyme-linked immunosorbent assay (ELISA) to assay the level of human aquaporin 4(AQP-4) in samples.

Laboratory investigations including complete blood picture, blood gases, pH determination with calculation of base deficit and creatinine phosphokinase (CPK) were done. The CPK was measured twice in the blood serum of hypoxic neonates and controls groups; one at two days of age and the second at seven days.

Computer Tomography (CT) was performed twice for patients groups; one at the two days of age and the second at seven days. +ve CT signs of HIE were narrowness of the lateral ventricles and flattening of gyri. Areas of reduced density that indicate evolving zones of infarction may be present. Evidence of hemorrhage in the ventricles or in the cerebral parenchyma may also be seen [18].Written informed consent was obtained from all participants. The study protocol was approved

by the research ethical committee of Faculty of Mmedicine, Zagazig University. Moreover, the study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:

Data were analyzed using Statistical Package for the Social Sciences (SPSS) program for Windows version 22. Quantitative data described using mean ± standard deviation; qualitative data described in the form of numbers and percentage. Student t-test was used when comparing two means. Chi-square used for comparison of qualitative variables. Analysis of variance (ANOVA of F test) was used for comparison of means of more than two groups. For all above statistical tests done, the results were considered significant when the probability of error is less than 5% ($p<0.05$).

The cut-off indicated by receiver operating curve (ROC) analysis was used to evaluate the positive and negative predictive values, specificity and sensitivity, with their respective 95% confidence bounds. Moreover, the correlation between each of serum AQP4and CPK levels with the severity of HIE was determined.

RESULTS

There was no statistically significant difference between patient group and control group in relation to gestational age, sex and weight (Table 1). There was a statistically significant increase in white blood cell count in HIE group than the control one. The aquaporin-4 at age of 2 days (AQP4-1) was found to be significantly higher in hypoxic patients than the control (220.83±98.85 vs. 135.51± 28.32 pg/ml). Moreover, the level of AQP4 at age of 7 days (AQP4-2) was higher than its level at age of 2 days and the value was about 349.72 ±110.25 pg/ml. There was a significant increase in CPK-1and CPK-2 levels in HIE group compared to the control. There was a highly significant poor arterial blood gases in HIE group (pH <7.0 or base deficit ≥12 mmol/l) in comparison to the control which had good arterial blood gases. There was a highly significant decrease in Apgar score in HIE group at 1 and 5 minutes.

In Table 2, at age of 2 days, there was no significant difference in AQP-4 level between preterm and full term hypoxic patients. Also, there was no significant difference in AQP-4 level between male and female hypoxic patients. The serum AQP-4 level was significantly higher in patients with convulsion (283.83 ± 65.01 pg/ml) in comparison to patients without convulsion (127.04±47.50 pg/ml). Moreover, it was found that the patients necessitating more anticonvulsant drugs for control of seizures had significantly higher serum AQP-4 levels. The AQP-4 level was found to be significantly higher in patients with positive CT changes (251.58±90.13 pg/ml) in comparison to patients with negative CT (181.284±94.55 pg/ml). In addition, the severe hypoxic patients had significantly higher AQP4 levels than other hypoxic patients, and the moderate hypoxic cases had higher AQP4 values than the mild patients. The CPK levels were observed to be significantly higher in moderate and severe hypoxic patients than the mild cases.

In Table 3, at age of 7 days, there was a significant increase in AQP-4 level in preterm than full term hypoxic patients. However, there was no significant difference in AQP-4 level between male and female hypoxic patients. The serum AQP-4 level was significantly higher in patients with convulsion (420.21±86.06pg/ml) in comparison to patients without convulsion (243.01±22.80 pg/ml). In addition, it was found that patients necessitating more anticonvulsant drugs for control of seizures had significantly higher serum AQP-4 levels. The AQP-4 level was found to be significantly higher in patients with positive CT signs of HIE (384.46±110.26 pg/ml) in comparison to patients with negative CT signs of HIE (308.08±93.62 pg/ml). Moreover, the severe hypoxic patients had significantly higher AQP4 levels than other patients, and the moderate hypoxic cases had higher AQP4 values than the mild ones. The CPK levels were found to be significantly higher in severe hypoxic patients than the moderate or mild cases, and the moderate cases had higher CPK values than the mild ones.

The risk factors of HIE is shown in Table 4, which were AQP4-1 >158 pg/ml, platelets < 150 000 $x10^3/mm^3$, gestation age > 40 weeks, and Apgar < 5. Table (5) showed that the best cut- off for AQP4 to detect the hypoxia was >158 pg/ml with a sensitivity of 66.7%, specificity of 73.3%, positive predictive value of 75.0% and negative predictive value of 6.5%. Thus, the serum AQP4 level can be used in the diagnosis of HIE as it has high area under the curve.

Table 1: Demographic, clinical and laboratory characteristics of the studied groups

Variables	Patients (n = 30)	Controls (n = 30)	t / x^2	*p*- value
Gest. age (weeks)				
X ± SD	39.03 ±2.13	38.8 ± 0.89	0.56	0.58
Range	32-41	37 – 40		

Variables	Patients (n = 30)	Controls (n = 30)	t / x^2	*p*- value
Gender (No, %)				
Male	17 (56.7%)	17 (56.7%)	0.53	0.60
Female	13 (43.3%)	13 (43.3%)		
Weight (in kg)				
X ± SD	3.09 ± 0.71	2.93 ± 0.22	1.23	0.22
Range	1.5 – 5.0	2.5 - 3.2		
White blood cell count				
(X 10^3 mm^3)	10.95 ± 5.70	6.83 ± 0.69	3.93	<0.001
AQP4 -1 (pg/ml), at age of 2 d				
X ± SD	220.83±98.85	135.51± 28.32	4.63	<0.001
Range	68.7- 385.5	54.4 – 169		
AQP4 -2 (pg/ml), at age of 7 d				
X ± SD	349.72±110.25	n.d*	-	-
Range	210 – 557			
CPK-1 (U/L), at age of 2 d				
X ± SD	200.62±78.53	147.67± 60.48	2.78	0.007
Range	111 - 378.9	68 – 269		
CPK-2 (U/L), at age of 7 d				
X ± SD	284.7±115.4	n.d*	-	-
Range	141 - 554			
ABG-1, at age of 2 d				
pH	6.80 ± 0.14	7.33±0.05	5.43	<0.001
Base deficit (mmol/l)	14.2 ±7.2	4.0 ±0.11	6.23	<0.001
ABG-1, at age of 7 d				
pH	7.13 ± 0.12	n.d*	-	-
Base deficit (mmol/l)	18.3 ±7.2			
Apgar sore –at 1 min.				
X ± SD	1.55 ± 0.90	7.30 ± 1.10	4.55	<0.001
Apgar sore –at 5 min.				
X ± SD	5.0 ± 1.54	9.0 ± 1.02	5.07	<0.001

*n.d: non determined. CPK: creatine phosphokinase. ABG: arterial blood gases.

Table 2: Mean AQP4 (measured at age of 2 days) serum levels (pg/ml) in HIE patients, with measured CPK values

	Patients (n = 30)	Test of significance (t, f)	*p*- value
Gestation age			
Preterm	275.33 ± 69.72 (200 - 337.6)	1.05 t	0.30
Full term	214.55 ± 97.06 (78.8 -383.4)		
Gender			
Male	229.72 ± 84.44 (87.7 - 383.4)	0.55 t	0.59
Female	209.86 ± 114.26 (68.7 - 385.5)		
Convulsion			
No	127.04 ± 47.50 (68.7 - 228.6)	7.16 t	<0.001
Yes	283.83 ± 65.01 (118.8 - 383.4)		
Number of anti-convulsant			
0	127.04[c] ± 47.50 (68.7 - 228.6)	20.3 f	<0.001

	Patients (n = 30)	Test of significance (t, f)	*p*- value
1	239.00[b] ± 83.60 (118.8 - 341.4)		
2	295.39[ab] ± 56.57 (229.3 -385.5)		
3	313.88 [a] ±33.79 (264.3 - 337.6)		
CT			
- ve	181.28 ± 94.55 (87.7 - 383.4)	2.07 t	0.047
+ ve	251.58 ± 90.13 (68.7 - 385.5)		
Degree of HIE			
Mild 12 cases X±SD (range)	127.04[c] ± 47.50 (68.7 - 228.6)	31.4 f	<0.001
Moderate 12 cases X±SD (range)	263.82[b] ± 67.20 (118.8 - 385.5)		
Severe 6 cases X±SD (range)	323.87[a] ± 39.37 (264.3 - 383.4)		
CPK			
Mild 12- HIE cases	176.02[b]±75.10 (111 - 358)	45.2 f	0.032
Moderate 12- HIE cases	219.90[a]±86.29 (119 - 378.9)		
Severe 6- HIE cases	214.50[a]±68.77 (119.4 - 322)		

Table 3: Mean AQP4 (measured at age of 7 days) serum levels (pg/ml) in HIE patients, with measured CPK values

	Patients (n = 30)	Test of significance (t, f)	*p*- value
Gestation age			
Preterm	453.93 ± 66.19 (400- 572.8)	1.77 t	0.087
Full term	339.93 ± 108.22 (196.9 -557)		
Gender			
Male	354.57±106.49 (196.9 - 557)	0.29 t	0.77
Female	342.47±120.82 (210 - 557)		
Convulsion			
No	243.01± 22.80 (196.9 - 275.8)	6.94 t	<0.001
Yes	420.21± 86.06 (238 - 523.4)		
Number of anti-convulsant			
0	243.01[c]± 22.80 (196.9 - 275.8)	29.6 f	<0.001
1	373.32[b] ± 62.44 (275.6 - 434)		
2	398.25[b] ± 73.93 (238 - 523.4)		
3	524.70[a] ± 47.19 (457 - 557)		
CT			
- ve	308.08 ± 93.62 (233 - 523.4)	2.0 t	0.050
+ ve	384.46 ± 110.26 (210 - 557)		
Degree of HIE			
Mild 12 cases X±SD (range)	243.01[c]±22.80 (210 275.8)	77.1 f	<0.001
Moderate 12cases X±SD(range)	373.55[b]±58.21 (238 - 434)		
Severe 6 cases X±SD (range)	513.52[a]±45.30 (457 - 458.9)		
CPK			
Mild 12- HIE cases	196.4[c]±47.10 (141 - 300)	38.5 f	0.021
Moderate 12- HIE cases	295.3[b]±87.3 (180 - 433)		
Severe 6- HIE cases	442.0[a]±75.9 (367 - 554)		

Table 4: Risk factors of HIE

	HIE *N %*	Control *N %*	OR (95% CI)	*p*- value
AQP4-1 (pg/ml)				
>158 (pg/ml)	20 (66.7%)	8 (26.7%)	3.07	<0.001
<158 (pg/ml)	10 (33.3%)	22 (73.3%)	(1.30-4.84)	
Platelets count (x10^3/mm^3)				
≤ 150 000	12 (40%)	0 (0%)	Undefined	0.04
≥ 150 000	18 (60%)	30 (100%)		
Gender				
Male	17(65.7%)	17 (65.7%)	0.21	0.60
Female	13(43.3%)	13(43.3%)	(1.61-2.03)	
Gestational age (weeks)				
> 40	5 (16.7%)	0 (0%)	Undefined	0.32
≤ 40	25 (83.3%)	30 (100%)		
Apgar (1 min)				
< 5	6 (20%)	0 (0%)	Undefined	0.40
> 5	24 (80%)	30 (100%)		

Table 5: Indices of diagnostic accuracy of AQP4 for prediction of HIE

Cut off	With	Without	Sensitivity	Specificity	Predictive value +ve -ve	AUC (95% CI)
>158 (pg/ml)	20	8	66.7%	73.3%	75.0% 6.5%	0.714 (0.568 - 0.860)
<158 (pg/ml)	10	22				

Accuracy 95%.

AUC, area under the receiver operator characteristic (ROC) curve; CI, confidence interval.

DISCUSSION

Hypoxic ischemic encephalopathy after perinatal asphyxia is an important cause of neonatal morbidity, neurological disability & mortality. The early prediction of hypoxic ischemic encephalopathy is particularly important because of the brief therapeutic window and possible side effects of neuro protective interventions [19]. In the present study, 65.7% of our patients were males, while 43.3% were females, and this result was no significant difference between HIE and male gender, and this result concur with that of Albanna and Ahmed [21] who studies the risk factors of HIE in 20 neonates and found out that there was no significant difference between HIE and male gender, in contrast to Itoo et al. [22] and Futrakul et al. [23] who studied the risk factors of HIE in 84 neonates and found out that there was a statistically significant relationship between HIE and male gender; this may be due to higher number of patients in these studies.

In the current study, the patients group had a mean weight of 3.09 ± 0.71kg and a mean gestational age 39.03±2.13weeks, while in the control group, the mean weight was 2.93±0.22 kg and mean gestational age of 38.8±0.89 weeks. There was no statistically significant difference between the patients group and the control group in relation to weight and gestational age. This is in agreement with the result of Guo et al. [24].

The aquaporin-4 at age of 2 days was found to be significantly higher in hypoxic patients than the control (220.83±98.85 vs. 135.51± 28.32 pg/ml). Moreover, the level of AQP4 at age of 7 day was about 349.72±110.25 pg/ml. These findings coincide partially with the results of Clement et al. [10] who reported that AQP4 plays an important role in mediating brain edema in hypoxic-ischemic encephalopathy. Moreover, it was found that there was no significant difference in AQP-4 level between preterm and full term hypoxic patients at age of 2 days, as the values were 275.33±69.72 pg/ml for preterm and 214.55±97.06 for full term. While, there was a significant increase in AQP-4 level in preterm than full term hypoxic patients at age of 7 days, as the values were 453.93±66.19

pg/ml for preterm and 339.93±108.22 pg/ml for full term. This result agrees with that of Krishnan P, Shroff [25] and Bano et al. [26]. In addition, there was no significant difference in AQP-4 level between male and female hypoxic patient at age of 2 days, as the values were 229.72±84.44 pg/ml for male and 209.86±114.26 pg/ml for female, also there was no significant difference in AQP-4 level between male and female hypoxic patient at age of 7 days, as the values were 354.57±106.49 pg/ml for male and 342.47±120.82 pg/ml for female. These findings agree with that of Douglas-Escobar and Weiss [5] and Zhu et al. [27]. However, at age of 2 days, the serum AQP-4 level was significantly higher in patients with convulsion (283.83±65.01 pg/ml) in comparison to patients without convulsion (127.04±47.50 pg/ml). Also, at age of 7 days the serum AQP-4 level was significantly higher in patients with convulsion (420.21 ± 86.06pg/ml) in comparison to patients without convulsion (243.01±22.80 pg/ml). These findings are supported with the results of Irene et al. [28] and Oklinski et al. [29].

In the present study, the Apgar score of HIE group at 1 and 5 minute showed a mean of 1.55 ± 0.90 at 1st minute and 5.0 ± 1.54 at 5 minutes, while in the control group the mean of Apgar score at 1 and 5 minutes was 7.30 ± 1.10 and 9.0 ± 1.02, respectively. The HIE group showed a significantly low Apgar score than the control. This result is in agreement with that of Hassanein et al. [34], who made a study on 38 newborn with hypoxic ischemic encephalopathy and showed a significantly low Apgar score at 1, 5, and 10 minutes (0.47, 3.15 and 6.1) in comparison to the control group which had Apgar score of 6.63, 9.0 and 9.56 at 1, 5 and 10 minutes, respectively. These results agrees with the findings of Belai et al. [35] and Killion [36], they confirmed that low Apgar score was associated with an increased risk for HIE.

There was a statistically significant difference between the HIE group and the control group in white blood cells count. This result concurs with that of Boskabadi et al. [37]. Moreover, the HIE group had poor umbilical cord gases (pH < 7.0 or base deficit ≥12 mmol/l) in comparison to the control which had good umbilical cord gases, thus, there was statistically significant difference between the HIE group and the control group. This result coincides with that of Kimberly et al. [32].

In the present study, 40% of our patients had platelets count ≤ 150000 $x10^3/mm^3$, while 60% of the patients had platelets count ≥ 150000 $x10^3/mm^3$ in comparison with the control group which had 100% of platelets count ≥ 150000 $x10^3/mm^3$. This finding showed that there was a statistically significant relationship between the HIE patients and the control group. This result agrees with that of Albanna and Ahmed [21] and Boskabadi et al. [37].

In the present study, at age of 2 and 7 days, it was found that patients necessitating more anticonvulsant drugs for control of seizures had significantly higher serum AQP-4 levels. This result is supported by the findings of Yozawitz et al. [30]. Moreover, at age of 2 days or 7 days, the AQP-4 level was found to be significantly higher in patients with positive CT signs of HIE in comparison to patients with negative CT. These results coincide with that of Saadoun and Papadopoulos [9] and Chu et al. [31]. At age of 2 days or 7 days, it was found a positive strong correlation between the blood level of AQP-4 and the severity of HIE with a value of 0.814 at 2 days of age and 0.922 for 7 days. These findings agree with that of Kimberly et al. [32] and Studer et al. [33]. Our study detected that the cut-off value of serum level of AQP-4 was 158 pg/ml, with sensitivity of 66.7%, specificity of 73.3%, and 95% of accuracy. There was a significant increase in the serum CPK level in the HIE neonates than the control group. This is in agreement with that of Hassanein et al. [34] , who showed that the mean value of total CPK 347.3 ± 162.5 U / L in 38 hypoxic ischemic neonates with a significant difference between the patient and control groups. The correlation value between CPK measured at 2 days of age and the severity of HIE was 0.223, but it was 0.802 at 7 days of age. Moreover, the correlation between AQP4 and CPK was found to be 0.401 at 2 days of age, and 0.803 at 7 days of age.

CONCLUSIONS

The obtained results indicate that the hypoxia leads to an increase in serum Aquaporin-4 level and total CPK enzyme activity. It can be concluded that the serum Aquaporin-4 level and total CPK enzyme activity could be used as early predictors in diagnosis of hypoxic ischemic encephalopathy. Combined detection of serum Aquaporin-4 level and total CPK enzyme activity in differentiation between grades of hypoxia gives us better sensitivity and specificity than each other.

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