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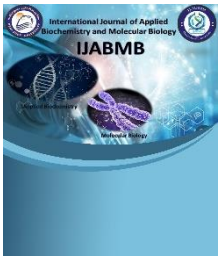

Diagnostic Utility of Aquaporin-4 blood level in Neonatal Hypoxic Ischemic Encephalopathy

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Running Title: Aquaporin-4 in Neonatal Encephalopathy.

Abstract:

Background: hypoxic ischemic encephalopathy is one of the most important diseases in neonates.

Objective: to evaluate the use of the blood level of aquaporin-4 in diagnosis of hypoxic ischemic encephalopathy in neonates, and to determine the severity of hypoxia.

Methods: case control study, carried out on 40 hypoxic neonates, sample (1) at age of 2 days while sample (2) at age of (7) days and 40 samples from healthy control. The collected blood samples were used to measure the aquaporin-4 level. Clinical neurological examination was done to detect patients with HIE.

Results: there was a statistically significant higher increase of AQP4 in cases than controls and a significant increase of it from 2nd day to 7th day, all cases had delayed crying after birth and most of them were hypotonic and most of them had convulsions, most of cases had convulsions highly significant correlation between Aquaporin 4 level and the severity of ischemic hypoxic encephalopathy in neonates either at 2 or 7 days of age.

Conclusion: it is found that, hypoxia leads to an increase in serum Aquaporin-4 level, serum Aquaporin-4 level could be used as early predictor in diagnosis of hypoxic ischemic encephalopathy, and serum Aquaporin-4 level in differentiation between grades of hypoxia gives us better sensitivity and specificity.

Keywords: Aquaporin-4, Hypoxia, Neonates, Encephalopathy, Ischemia.

Introduction:

Aquaporin-4 (AQP-4); a member of Aquaporins family, it's present in enormous level in the brain and spinal cord, where it's markedly polarized, with marked expression in the end-feet of the astrocytes that envelop capillaries (1). Following cerebral injury, AQP-4 was documented to be overexpressed in astrocytes. AQP4 action is to organize H2O alterations inside and out of the parenchymal tissues of the brain (2).

In neonates, cerebral hypoxic ischemia constitutes the principal reason of brain edema that leads to neurodevelopmental disturbed function (3).

The methods used to diagnose HIE are more strenuous, costly, expensive, and usually harm patients, but diagnosing HIE by measuring the AQP4 in blood is faster, costless and safe [4]. Currently, no research is carried out about the use AQP4 serum level in diagnosing HIE in human neonates at Pediatrics Departments, Faculty of Medicine, Beni-suef University. Thus, this thesis may throw light on possibilities for novel laboratory assessment of asphyxia. Furthermore, the study tried to assess blood levels of AQP4 in diagnosing neonatal HIE.

Patients and Methods

The study was performed at NICU, Pediatrics's Department, Beni-Suef Hospital, and at Pediatrics and Medical Biochemistry Departments, Faculty of Medicine, Beni-Suef University. Approval was obtained from the ethical committee in pediatric department, Beni-Suef University and from the patients included in the study. Approval number is (FMBSUREC/10102021/Taha).

The sample was a comprehensive sample from patients admitted in NICU, Pediatrics's Department, Beni-Suef Hospital. The blood samples were collected from 40 hypoxic neonates, sample (1) at the 2nd day of age while sample (2) at 7th day of age and 40 samples from healthy control neonates.

Criteria of inclusion: This case control study was carried out on: Cases: neonates, both inborn and out born, admitted to NICU, at Pediatrics's Department, Beni-Suef Hospital, diagnosed as HIE according to the AAP and the ACOG as non-reassuring fetal status (bradycardia, late deceleration of the fetal HR, marked and repeated variable deceleration of the fetal HR, decreased beat-to-beat variability). Asphyxia was defined based on an Apgar score <3 at the 5th min, pH < 7.0, or BE <12 in cord blood or venous blood taken from newborns within 60 min of birth, or the need for PPV (> 3 min) (5). Infants who fulfilled ≥3 of the above clinical and biochemical criteria and developed HIE, as defined by Levene staging (6), and 13 were delivered by emergency cesarean section and 17 were delivered vaginally. Controls: were 40 healthy neonates at the same gestational age, 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.

Criteria of exclusion: Neonates with any malformation, systemic infection, IUGR, or cardiac or hemolytic diseases were excluded from the study. Other exclusion criteria were multiple pregnancies, history of neurologic diseases in the family, any consanguinity, congenital or peri-natal infection such as chorioamnionitis, and maternal drug addiction, HTN, or DM.

Hospital protocol was used to manage HIE neonates in NICU. They were given O2, IV fluids, vit K, inotropes (Dopamine and/or Dobutamine each by 1–20mg/kg/min) and anti-convulsant agents (Phenobarbitone 20 mg/kg as loading dose, followed by 3–5 mg/kg daily, and phenytoin was also added with same dose in non-responder to phenobarbitone), whenever needed.

All studied neonates subjected to history taking that included maternal history and delivery history lay stress on history suggesting perinatal asphyxia, Apgar score of newborns was assessed at 1st, 5th and 10th minutes and they were resuscitated according to NRP guidelines (7). Clinical assessment included general examination, gestational age estimation via new Ballard score (8), vital signs, BW and head circumference and neurologic examination within the 1st 24 h following birth and for 7 days to classify, of HIE based on the criteria Sarnat and Sarnat (9). The neonatologist evaluated the consciousness level, activity, posture, tone, neonatal reflexes, convulsions, number of anti-convulsant agents used where no, one and two anti-convulsant were used in 10%, 65% and 25% of our patients respectively.

Laboratory investigations that included CBC, ABG and pH determination with calculation of base deficit. Computer Tomography (CT) was carried out 2 times for patients' groups; the 1st at the 2nd day of age and the 2nd at the 7th day. +ve CT signs of HIE were narrowness of the lateral ventricles and flattening of gyri. Areas of decreased density that indicated evolved zones of infarction along with evidence of intraventricular hemorrhage or in the cerebral parenchymal tissues were assessed as well (10).

Measurement of blood level of aquaporin 4: The blood samples (2 ml) were collected 2 times in the hypoxic neonates and controls groups, the 1st at the 2nd day of age and the 2nd at the 7th day. These blood samples were collected in plastic tubes, and centrifugation at 3000 rpm for 15 min was carried out. Thereafter, the serum was separated in Eppendorf tubes and stored at – 20 °C until the chemical analyses by Human AQP-4 kit. The Kit used double-Ab sandwich ELISA to assay the level of human AQP-4 in samples by adding AQP-4 to monoclonal antibody enzyme to the wells that were pre-coated with human AQP4 monoclonal Abs labeled with biotin and combined with streptavidin-HRP to form immune complexes,

then incubation and washing were done for removing uncombined enzymes. Thereafter, addition of chromogen solution, A, B, the color of the liquid altered to bluish, and on adding acid, the color turned yellowish. The chroma of color and the level of the AQP-4 of sample were positive correlated.

Results:

Table 1 showed that there was no significant difference between cases and controls regarding their baseline characteristics (P-value>0.05).

Table 1: Baseline characteristics of the studied groups

Variables	**Patients (n = 40)**	**Controls (n = 40)**	***p*-value**
Age (days)			0.063
MEAN ± SD	1.4±0.7	1.8±1.1	
Range	1-3	1 – 5	
Gender (No, %)			0.823
Male	19(47.5%)	20(50.0%)	
Female	21(52.5%)	20(50.0%)	
Weight (in kg)			
MEAN ± SD	2.8±0.8	2.9±0.7	0.253
Range	1.2 – 3.7	2- 4.1	
Maturity			>0.999
Mature	11(27.5%)	11(27.5%)	
Immature	29(72.5%)	29(72.5%)	
Consanguinity			0.366
Negative	21(52.5%)	25(62.5%)	
Positive	19(47.5%)	15(37.5%)	

Table 2 showed that 22.7% of cases needed MV. Nineteen cases died and 21 discharged.

Table 3 showed that there was a statistically significant higher increase of AQP4 in cases than controls. In addition, there was a significant increase of it from 2nd day to 7th day (P-value<0.001).

Table 4 showed highly significant (P< 0.001) correlation between AQP4 level and the severity of HIE in neonates either at 2 or 7 days of age. The correlation values were 0.712 and 0.553 at age of 2 and 7 days, respectively.

Table 5 showed that there was a significant moderate linear positive correlation between the NICU length of stay and AQP4 at 2nd and 7th day of admission (P-value<0.001).

Table 6 showed that there was a significant association between higher AQP4 level and immaturity at day 2 (P-value<0.05).

Table 7 showed that there was a significant association between higher AQP4 level and immaturity at day 7 and higher number of anticonvulsive drugs (P-value<0.05).

Table 8 showed that the best cut- off for AQP4 at 2nd day to detect the HIE was >133 pg/ml with a sensitivity of 100%, specificity of 97.5%, positive predictive value of 97.6% and negative predictive value of 100%. Thus, the serum AQP4 level can be used in the diagnosis of HIE as it has high AUC. In addition, the best cut- off for AQP4 at 7^{th} day to detect the HIE was >209 pg/ml with a sensitivity of 100%, specificity of 100%, positive predictive value of 100% and negative predictive value of 100%. curve.

Table 2. Need to MV, ICU admission, CT, MRI findings among the studied cases and their fate:

Examination	**Values** **No %**
MV	11-27.5
Duration of MV (mean±SD) **Range (min-max)**	7.1±3.3 (2-16)
Duration of NICU admission (mean±SD) **Range (min-max)**	12.5±6.8 5-33
CT brain **Normal** **Moderate HIE**	 22-55.0 18-45.0
MRI **Normal** **Mild HIE** **Moderate HIE** **Severe HIE**	 33-82.5 5-12.5 1-2.5 1-2.5
Prognosis **Died** **Discharged** **Without drugs** **With one drug**	 19-47.5 21-52.5 13-61.9 8-38.1

Table 3. Comparison between cases and controls regarding the Aquaporin 4 serum level Pg/mL at 2nd and 7th days:

Variables	Patients (n = 40)	Controls (n = 40)	*p*-value
AQP4 (pg/ml), at age of 2 d			
MEAN ± SD	280.8±67.7	99.1±24	<0.001*
Range	189- 487	66 – 209	
AQP4 (pg/ml), at age of 7 d			
MEAN ± SD	867.1±140.3	NA	-
Range	609 – 1210		
P-value between 2nd and 7th day	<0.001*		

*P-value is significant NA: not applicable

Table 4. Correlation between AQP4 and severity of HIE

	Correlation value	*p*- value
AQP4-1 vs HIE at 2nd day	0.711	< 0.001*
AQP4-2 vs HIE at 7th day	0.553	< 0.001

Table 5. Correlation between AQP4 at 2nd and 7th day and age, weight, MV duration and NICU length of stay:

		2nd day	7th day
Age Day	Pearson Correlation (r)	-.152	-.211
	P-value	.177	.061
Weight (kg)	Pearson Correlation (r)	.066	-.054
	P-value	.564	.632
duration days	Pearson Correlation (r)	.271	-.016
	P-value	.223	.943
Nicu stay duration days	Pearson Correlation (r)	.596**	.542**
	P-value	<0.001	<0.001 *

*P-value is significant

Table 6. Relation between different patients' characteristics & AQP4 at day 2

	Patients (n = 40)	***p*- value**
Gender		
Male	272.5±80.1	0.468
Female	288.3±55.2	
Maturity		
Mature	232.1±49.5	0.004*
Immature	299.3±65	
Convulsion		
No	248±35.3	0.488
Yes	282.6±68.8	
Number of anti-convulsant		
0	285±84	0.107
1	255.5±56.5	
2	297.6±57.3	
3	318.1±78.7	
CT		<0.001*
Normal	110.2±13	
Positive (HIE changes)	170.6±16	

*P-value is significant

Table 7. Relation between different patients' characteristics & AQP4 at day 7.

	Patients (n = 40)	***p*- value**
Gender		
Male	847.8±135.5	0.418
Female	884.4±145.5	
Maturity		
Mature	790.6±95.3	0.032*
Immature	896.1±144.9	
Convulsion		
No	837±82.0	0.760
Yes	868.6±143.2	
Number of anti-convulsant		
0	800±166.8	0.037*
1	817.2±96.2	
2	879.5±125.5	
3	965.5±173.6	
CT	867	<0.001*
Normal	352±67	
Positive (HIE changes)	515±82	

*P-value is significant

Table 8. Indices of diagnostic accuracy of AQP4 for prediction of HIE

	AQP4 at 2nd day	**AQP4 at 7th day**
AUC	0.996	1.000
P-value	<0.001*	<0.001*
Cut off	>133	>209
Sensitivity	100.00(91.2 - 100.0)	100.00(91.2 - 100.0)
Specificity	97.50(86.8 - 99.9)	100.00(91.2 - 100.0)
PPV	97.6(85.2 - 99.6)	100.00(91.2 - 100.0)
NPV	100.00(91.2 - 100.0)	100.00(91.2 - 100.0)
Accuracy	98.7%	100%

Discussion

The term HIE refers to the neurological syndrome that occurs in newborn infants subjected to various degrees of a hypoxic-ischemic event. It's accompanied by lost consciousness; decreased of spontaneous movement, tone, and various reflexes; and the development of convulsions in severe cases (11).

various disorders are accompanied by HIE, convulsions, CP, attention deficits, hyperactivity disorders, fits, hearing and visual loss, language disorder, and delayed cognitive functions. The different outcome of such condition is marked, greatly influencing the patient's & his/her family's daily life. In fact, HIE is considered a major economic burden (12).

Neonatal HIE arises due to an insult involving a duration of decreased blood flow (ischemia) and O2 delivery to the neonatal brain (hypoxia) that might result from placental abruption, rupture of the uterus, and cord prolapse. Such condition consists of various stages: energy depletion, inflammatory status, excitotoxicity, OS, and cell death. Months following the hypoxia-ischemia insult, changes that occur as a result of this insult continuously occurs, such as late apoptosis, remodelling of the affected brain, astrogliosis, in addition to epigenetic alterations (13).

AQP4 is the principal membrane H2O channels present in the brain responsible for regulation of water homeostasis and is markedly expressed in the perivascular domain of astrocytes as well as the subpial domain of ependymal cells, where it's involved in H2O exchange between the brain tissue, circulating blood, and CSF. It has a pivotal role in CSF homeostasis, and changes in the expression of such type of aquaporins is accompanied by hydrocephalus (14).

In fat, scant studies concerning the importance of neonatal AQP4 in the diagnoses HIE, therefore, the aim of the present study was to investigate the utility of using the blood level of AQP4 in diagnosing HIE in neonates, and to assess the relationship between the AQP4 level and the severity of HIE.

This prospective case control study included 40 neonates (mean age 1.4±0.7 days) who were diagnosed as HIE (HIT) and a control group (mean age 1.8±1.1 days) that included 40 neonates admitted without HIT. Cases were recruited and assessed for eligibility from NICU, Pediatric Department. Beni-Suef University Hospital, Faculty of Medicine.

Regarding the neurologic assessment of the studied HIE patients, the present study revealed that all cases had delayed crying following birth, 77.5percent of them have hypotonia, 35 percent of them have absent reflexes, 25.5 percent of them have sluggish reflexes and 95 percent of them had convulsion. Convulsions were frequent in 42.1 percent of cases.

Such findings agreed with a case–control study by Abd El-Halim et al. [15] that compared 15 babies having HIE and 15 healthy control and concluded that consciousness, Moro& suckling reflexes, and tone were statistically significantly reduced among cases than the controls.

Similarly, Namusoke et al. (16) study on 23 HIE children revealed that nutritive suckling reflex was absent (nasogastric feeding), poor Moro reflex, and needing ventilation support (O_2) therapy via nasal prongs) were the common complications by the 7th day.

However, Goswami et al. (17) study on 696 neonates documented fewer cases with convulsions (twenty percent) that might be because of recruitment of babies suffering mild HIE only.

The present study revealed that 27.5 percent of cases needed MV and the mean duration of the MV was 7.1±3.3 days. The mean duration of NICU admission was 12.5±6.8 days.

Similarly, Goswami et al.(17) study on neonates suffering mild HIE managed according to the standard care (N = 696) concluded that 29 percent at the standard care group required MV, while, less duration of MV (1 (range: 0–2) days) and hospital stay (6 (range: 4–9) days) was demonstrated that might be explained to excluding moderate & severe cases of HIE in their study.

Less need for MV was reported by Hemeda et al. (18) study on 26 neonates with suspected HIE demonstrated that 15.4 percent of cases required MV, 19.2 percent of cases needed neonatal resuscitation and 26.9 MV developed symptomatic convulsions. Furthermore, less duration of hospital stay was documented by Debnath et al. (19) that studied 60 HIE cases and exhibited that their mean duration of NICU stay was 7.19 ± 5.26 days.

Regarding prognosis, the present study denoted that 47.5 percent of cases have been died and 52.5percent were discharged, 61.9percent of them were discharged with no need to prescribe medications and 38.1percent were prescribed with one drug.

Less mortality rate among HIT babies was documented by Talat et al. (20) that compared 60 HIT babies with 60 healthy control and showed that the mortality rate among HIT neonates was twenty percent (12 cases (four of them in stage II, 8 in stage III) from 60 asphyxiated babies). Moreover, a case-series study by El-Halim (21) on the complications of management of HI in neonates via the use of therapeutic hypothermia revealed that among the 14 cases with HIE, survivors were 71.6 percent and mortality represented 28.6 percent of cases. Additionally, Basiri et al. (22) study on 51 babies with asphyxia who were subjected to therapeutic hypothermia via the use of selective head cooling exhibited the death of 16 babies (31percent).

Furthermore, a retrospective study by Beltempo et al. (23) of children admitted for HIE and managed via therapeutic hypothermia in 24 tertiary NICUs from the Canadian Neonatal Network reported that out of the 3261 HIE cases, 367 (11 percent) died (range 5–17 percent) and 1033 (37 percent) of the 2822 with MRI findings confirmed brain insult (range 28–51%).

A prospective cohort study by Abdalla et al. (24) on 35 full term (more than 35 weeks) infants suffering HIE exhibited that elevated grades of HIE were accompanied by mortality that frequently greater than those with mild or moderate level of HIIE, also, there were statistically significant relationships between mortality and brain edema and that incidence of mortality was statistically elevated in neonates with grade III compared to those with grade I & II (represented 83.3 percent Vs. 0 percent and 35 percent respectively).

Namusoke et al. (25) reported that out of the 23 cases who got HIE, 6/23 (26 percent) died. Two thirds (2/3) were grade III HIE, and the remaining (1/3) had grade II HIE. Most of cases, 15/23 (65.2 percent), were discharged with absent short-term complications, while 2/23 (8.6 percent) were still admitted with complications by the 7th day. The complications included: poor Moro reflex, absent nutritive suckling reflex (nasogastric feeding), and needing respiratory support (O_2 via nasal prongs). There were 7/23 (30 percent) with fits and these were controlled by the 5th day.

A previous study by Debnath et al. (19) on sixty HIE babies exhibited that most of cases (73.9 percent) of neonates with HIE were discharged with no short-term complications by one week.

However, during neurodevelopmental evaluation, only 11.67 percent of neonates showed normal development while development was mild, moderately and severely affected in ten percent, 33.33 percent and twenty percent babies respectively. Significant associations were determined between moderately to severely impaired/death with stage III of HIE.

Regarding the AQP4 blood level at the 2nd day, the present study revealed a statistically significant increase in mean AQP4 plasma levels among cases in comparison with controls (280.8±67.7 versus 99.1±24, respectively) (P value<0.001). Moreover, among cases group, the mean AQP4 serum levels at 7th day increased significantly in comparison with the AQP4 plasma level at the 2nd day (867.1±140.3 versus 280.8±67.7, respectively) (P value<0.001).

The present study revealed the existence of positive correlation between AQP4 at 2nd day and severity of HIE (r=0.711, P value<0.001) and the existence of positive correlation between AQP4 at 7th day and severity of HIE (r=0.553, P value<0.001).

That could be explained by Clément et al. (26) who concluded that AQP4 has pivotal roles in inducing brain oedema in HIE.

Such findings agreed with Elbanna et al. (27) that assessed the blood levels of AQP4 in thirty neonates with HIE and thirty babies not suffering HIE and demonstrated that the mean AQP4 at age of 2 days was found to be significantly elevated in hypoxic cases in comparison the control (220.83±98.85 vs. 135.51± 28.32 pg/ml). furthermore, the mean levels of AQP4 at 7^{th} day among cases group was further elevation reaching 349.72±110.25 pg/ml. furthermore, At age of 2nd or 7th day, it was found a positive strong correlation between the serum levels of AQP4 and the severity of HIE at the 2nd day (r= 0.814) and 7 days (r=0.922).

A previous study by Kitchen et al. (28) demonstrated that hypoxia induces a high AQP4 mediated flux of H2O into astrocytes. Translocation of AQP4 to the astrocytes surface driven increase in H2O flux.

Regarding the laboratory results of the studied cases, the present study revealed that the mean total Ca+2 among babies with HIE was 9.07 ± 0.67 mg/dL (range: 7.90-10.70 mg/dL) and the mean HCO3 was 17.51±4.58 (range: 10.30-28.00).

The present study showed the existence of moderate positive correlation between AQP4 at 2nd day and each of total Ca+2 (r=0.347, P value=0.038) and HCo3 (r=0.416, P value=0.008). nevertheless, at 7th day, no significant linear correlation was determined between the AQP4 and various laboratory and ABG parameters (P value>0.05).

A cross sectional study by Hassan et al. (29) on a total of one hundred full-term babies of both sex with evidence of birth asphyxia of any stage showed that the mean serum calcium levels were found to be 7.64 ± 0.59 mg/dl and that the hypoxic-ischemic encephalopathy stage stratification with mean blood Ca+2 level with high stages deciphered significant correlation (7.89 ± 0.54, 7.36 ± 0.28, and 6.88 ± 0.28 mg/dl, respectively, in stage I, II, and III) showing that a severely reduced level of mean blood Ca+2 were seen in higher hypoxic stages with HIE thus emphasizing a linear correlation with the beginning and progress of disease.

Another study by Mahajan et al. (30) on one hundred infants (50 HIE cases and 50 control subjects) demonstrated that total serum Ca+2 and ionic Ca+2 level at birth were significantly decreased in patients (8.04 ± 0.89 mg/dl, 3.62 ± 0.46 mg/dl) in comparison with controls (9.32 ± 0.72 mg/dl, 4.79 ± 0.49 mg/dl).

In the case group levels of total plasma Ca+2 and ionic Ca+2 level proved a decrease trend with increased stage of HIE. Levels of total plasma Ca+2 and ionic Ca+2 were 8.88±0.290, 4.03±0.178 mg/dl among babies who hadn't HIE, 8.07±0.675,3.61±0.354 mg/dl among babies who had HIE-1, 7.78±0.572, 3.54 ±0.572 mg/dl among neonates who had HIE-2 and 7.03±0.596, 3.12±0.342 mg/dl among neonates having HIE.

The present study revealed the existence of a significant moderate linear positive correlation between the NICU duration of stay and AQP4 at the second (r=0.596) and seventh days of admission (r=0.542) (P value<0.001) that might denote the increased NICU stay showed the more severe disease.

Similarly, a prospective study by Talat et al. (31) on 60 neonates who developed HIE revealed the existence of a highly significant relationship between long hospital stay and degree of HIE in asphyxiated babies as the hospital stay in grade I, II and III was 12.3±4.17 versus 25.0±7.9 vs. 37.25±6.18 days, respectively. A retrospective study by Flaten et al. (32) on 47 HIE neonates demonstrated that high NICU duration of stay and altered MRI findings might occur with more severe HIE in neonates born to mothers having greater BMI.

Moreover, Sarkar et al. (33) study on a total of ninety consecutively cooled HIE babies reported that the neonates with brainstem affection needed more prolonged hospital stay in comparison with infants without brainstem lesions (29 days, IQR 20–47 vs. 16 days, IQR 10–26, respectively).

The present study concluded that at the 2nd day, there were a statistically significant elevation in the mean AQP4 levels among immature babies in comparison with the mature ones (299.3±65 versus 232.1±49.5, respectively) (P value= 0.004). also, at the 7th day, the mean AQP4 was still significantly elevated among immature babies in comparison with the mature ones (896.1±144.9 vs. 790.6±95.3, respectively) (P value= 0.032).

Such results are partly in agreement with Elbanna et al. (27) that demonstrated a significantly elevated AQP4 level in preterm in comparison with full term hypoxic cases at the 7th day of age (453.93±66.19 versus 339.93±108.22 pg/ml, respectively) with decreased value of serum AQP4 in comparison with the present findings. nevertheless, there were no significant differences in AQP4 levels between preterm and full-term hypoxic cases at 2nd day of age (275.33±69.72 versus 214.55±97.06, respectively), which is in contrary with the present findings.

A cross-sectional study by Wang et al. (34) included 228 neonates showed that with the decrease in the neonatal BW, the detection rate of moderate or severe HIE in male and female babies showed gradual increase. Furthermore, the determination of moderate or severe HIE in male and female babies showed statistically significant increase with the decrease of neonatal gestational age at birth.

The present study revealed that at day 7, the mean AQP4 was significantly increased in infants took 3 anti-convulsion therapy in comparison with infants took 2 anti-convulsions in comparison with neonates took single anti-convulsion therapy compared with infants didn't receive any anti-convulsion drug (965.5±173.6 vs. 879.5±125.5 versus 817.2±96.2 versus 800±166.8, respectively).

Such findings agreed with Elbanna et al. (27) that demonstrated that the mean AQP4 was significantly increased in babies received three kinds of anticonvulsants in comparison with those received 1 type of anticonvulsants compared with neonates who did not receive anticonvulsant agents (524.70 ± 47.19 vs. 373.32 ± 62.44 vs. 243.01± 22.80, respectively) (P value<0.001).

A previous study by Dwivedi et al. (35) demonstrated that infants having moderate or severe abnormality of EEG showed poor response to phenobarbital as the non-responders had greater mean seizure score and markedly more injury on MRI scan for white matter, cortical parenchyma in addition to watershed areas. Thus, those infants need additional antiepileptic drugs.

Regarding the ROC curve for prediction of HIE using AQP4, the present study revealed that the AUC for AQP4 at day 2 was 0.997 with the best cutoff value > 133 with sensitivity of 100%, specificity =97.5 percent, and accuracy 98.7 percent. furthermore, as regards the ROC curve for predicting HIE by AQP4 at the 7th day present study revealed that the AUC for AQP4 at the 7th day was 1 with the best cutoff value greater than 209 with sensitivity , specificity , and accuracy were one hundred percent for all.

A previous study by Elbanna et al. (27) detected that the cut-off value of serum levels of AQP4 were 158 pg/ml, with sensitivity of 66.7 percent, specificity of 73.3 percent, and 95% of accuracy.

Conclusion

From the results, it is found that, hypoxia results in increased serum AQP4 level. It could be concluded that: Serum AQP4 levels might help in the differentiation between grades of hypoxia and its severity giving us better sensitivity and specificity.

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