SERUM GLIAL FIBRILLARY ACIDIC PROTEIN AS BIOMARKER IN NEONATES WITH HYPOXIC ISCHEMIC ENCEPHALOPATHY

By

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ABSTRACT

Introduction: *Hypoxic ischemic encephalopathy (HIE) is a potentially devastating condition accounts for 25% of all neonatal deaths , 40% will be affected by blindness, deafness, autism,, epilepsy, or other long-term complications(Felipe et al., 2013).*

Aim of work*: Assessment of the level of serum glial fibrillary acidic protein (GFAP) in neonate with hypoxic ischemic encephalopathy to early identify neonates with poor prognosis.*

Patients and Methods: *This study is a case-control study was carried out on 50 neonates babies, they were selected from (NICU) of Bab Elsharia hospital in Cairo during the period from October 2016 to March 2018.*

Results: *In our study we found that there was statistically significant correlation between GFAP at 24 hours age and this demonstrate a concentration-dependent relationship between serum GFAP at birth and the severity of encephalopathy (Chalak et al. (2014).*

Conclusion*: Serial increase in level GFAP from birth in HIE neonates and the severity of the hypoxic-ischemic injury can be stratified at birth and postnatal because elevated GFAP in serum correlated with severity of HIE.*

Recommendations: *Measurement of serum GFAP in HIE within 24 hours postnatal but with large sample to early identify neonates with poor prognosis.*

Key words: *Glial fibrilary acidic protein, hypoxic ischemic encephalopathy.*

INTRODUCTION

Defintion and Criteria for the diagnosis of HIE:

The standard for defining hypoxic ischemic event as sufficient to produce moderate to severe neonatal encephalopathy including:

The 4 essential criteria were:

(a) Metabolic acidosis (pH < 7.0).

(b) Moderate or severe encephalopathy in infants born at 35 or more weeks of gestation.

(c) Cerebral palsy of spastic quadriplegic or dyskinetic type.

(d) Exclusion of other etiologies (Roberto et al., 2014).

GFAP is the cytoskeleton structure of glia cells, it is structurally similar to other non-epithelial members (class III), including vimentin, desmin, and peripherin, and has a head, rod and tail domains (Biswas, 2011).

The function of GFAP is emerging evidence suggests that following traumatic brain and spinal cord injuries and stroke, GFAP protein and its breakdown products are rapidly released into biofluids, It is elevated in CSF and/or levels serum at 4 to 24 hour after injury making them strong candidate biomarkers for such neurological disorders (Zoltewicz et al., 2013).

The overall concept is that brain injury causes the release of GFAP-BDP and GFAP from injured astrocytes to the interstitial fluid extracellular fluid, where these proteins equilibrate into the subarachnoid CSF compartment, then release to the circulating blood by direct venous drainage or continue to follow the CSF flow and eventually enter the circulation by diffusing pass.

PATIENT AND METHODS

This study is a case-control study was carried out on 50 neonates babies , they were selected from (NICU) of Bab Elsharia hospital in Cairo during the period from October 2016 to March 2018 defined according to the following criteria It include two groups.

Group (1): The diseased group: 30 cases.

Inclusion criteria:

The 4 essential criteria were:

(a) Metabolic acidosis (pH < 7.0).

(b) Moderate or severe encephalopathy in infants born at 35 or more weeks of gestation.

(c) Cerebral palsy of spastic quadriplegic or dyskinetic type.

(d) Exclusion of other etiologies (Roberto et al., 2014).

Exclusion criteria:

Any newborn with one of the following:

- Chromosomal abnormalities or congenital malformations.
- Metabolic disorders.
- Congenital viral infections
- Septic shock.
- Preterm babies less than 37 weeks gestation (Chalak et al. (2014).

Sampling:

All patients were randomly rotated between both groups and undergo the following:

Thorough history includes:

1. Full history taking according to Bab Elsharia NICU clinical sheet.

- Prenatal history: for pre-existing maternal or fetal problems:
  - Maternal: e.g. hypertension, vascular disease, diabetes, drug use, Infection and vaginal bleeding.
  - Fetal: e.g. hydrops, and intrauterine growth retardation.
- Natal history: Documented history suggestive of perinatal asphyxia:
  - Abnormal uterine contractions, prolonged labor, mode of delivery, analgesia, anesthesia and amniotic fluid (normal, offensive or meconium stained).
  - Resuscitation by oxygen, ambu bag, endotracheal intubation, chest compressions, medications.
  - APGAR score at 1, 5 and 10 minutes (Apgar, 1953).
- Postnatal history: for pulmonary, cardiovascular or neurological abnormalities.

2. Thorough local and general clinical examination:

- Determination of the gestational age using Ballard scores (Ballard et al., 1991).
- Determination of birth weight, length and head circumference.
- Thorough clinical examination.
- Neurological examination including:
  - Level of consciousness.
  - Neonatal reflexes: e.g. suckling and Moro.
  - Presence or absence of seizures.
  - Sarnat staging and according to the criteria of Sarnat and Sarnat, HIE was classified as mild (Grade 1) if hyper-excitability, hyper-alertness, or hyper-reflexia persisted without seizures for at least 24 hours after birth; as moderate if the infant was lethargic, had hypotonia, weak primitive

reflexes, pupil miosis, and seizures; and as severe if the infant had apnea, flaccid weakness, frequent seizures, decelerated posture, or coma. (Sarnat and Sarnat, 1976).

● Chest, heart and abdominal examinations.

3. Laboratory Investigations including:

- Cord blood sample for blood gas immediately within an hour after birth (Hesham et al. (2005)).
- Complete blood count (CBC) (Automated analyzer).
- C reactive protein (Quantitative) Serum electrolytes: e.g. Na, K and Ca. -
- Serum urea and creatinine.
- ALT and AST. Random blood sugar.
- GFAP (glial fibrillary acidic protein) at 24 hours postnatal, using ELISA protocol.

Steps of research:

1. Approval of ethical committee of the department, college and university was obtained.
2. Informed consent was taken from all patients included in the study.
3. No conflict of interest in the study.
4. Venous blood samples about 2-4cm were taken serially from each patients at the first 24 hours post-natal and from the controller within the first hours postnatal.

Samples were allowed to clot upright at room temperature for 30 min in Processing lab, then spun at 1200RC at room temperature for15 min and separated.

Samples were measured GFAP level using a standard sandwich ELISA protocol. (ENZYMELINKED IMMUNOSORBENTASSAY)

RESULTS

Table (1): Comparison between the two studied groups regarding sex, gestational age and weight

		Control group	Cases group	Test value	P-value	Sig.
		No.= 20	No.= 30			
Sex	Female	10 (50.0%)	12 (40.0%)	0.487*	0.485	NS
	Male	10 (50.0%)	18 (60.0%)			
GA (wks)	Mean ± SD	37.85 ± 0.93	37.67 ± 1.32	0.537•	0.594	NS
	Range	37 – 40	36 – 42			
Wt (kg)	Mean ± SD	3.15 ± 0.35	2.93 ± 0.47	1.754•	0.086	NS
	Range	2.5 – 3.9	2.1 – 4			

•:Independent-test; *: Chi-square test
P-value > 0.05 Non-significant
P-value < 0.05 Significant

It shows that there was no statistically significant difference between the two studied groups regarding sex, gestational age and weight.

Table (2): Comparison between the two studied groups regarding history data of mothers and neonates

		Control group	Cases group	Test value	P-value	Sig.
		No.= 20	No.= 30			
Gestational AGE	Mean ± SD	32.45 ± 5.38	31.03 ± 7.21	0.749•	0.457	NS
	Range	22 – 40	19 – 45			
PARITY	Mean ± SD	2.45 ± 1.10	2.80 ± 1.61	-0.850•	0.400	NS
	Range	1 – 5	1 – 7			
Disease	HTN DM Hypotion		6 4 1	–	–	-
Mode of delivary	Normal	6 (30.0%)	17 (56.7%)	3.435*	0.064	NS
	CS	14 (70.0%)	13 (43.3%)			
Obstructed labour	Negative		9 (30.0%)	–	–	–
	Positive		21 (70.0%)			
APGAR At 5 min	Median (IQR)		6 (5 – 6)	–	–	–
	Range		4 – 7			
Conscious	Negative		9 (30.0%)	–	–	–
	Positive		21 (70.0%)			
Muscle tone	Flaccid	0 (0.0%)	4 (13.3%)	18.750*	0.000	HS
	Hypotonia	0 (0.0%)	14 (46.7%)			
	Normal	20 (100.0%)	12 (40.0%)			
Moro reflex	Negative	0 (0.0%)	19 (63.3%)	20.430*	0.000	HS
	Positive	20 (100.0%)	11 (36.7%)			
Convulsion	Negative		6 (20.0%)	–	–	–
	Positive		24 (80.0%)			
MAS	Negative		21 (70.0%)	–	–	–
	Positive		9 (30.0%)			
Respiratory distress	Negative	20 (100.0%)	1 (3.3%)	46.032*	0.000	HS
	Positive	0 (0.0%)	29 (96.7%)			

•: Independent-test; *: Chi-square test; ǂ: Mann-Whitney test
NS: Non significant; S: Significant; HS: Highly significant

It shows that there was highly statistically significant difference regarding neonatal data (muscle tone, moro reflex and respiratory distress) between the `q`we44eregarding maternal data (age, parity and mode of delivery) between the diseased and control group.

Table (3): Comparison between the two studied groups regarding ABG finding (PH, PACO2, PAO2, HCO3)

		Control group	Cases group	Test value•	P-value	Sig.
		No.= 20	No.= 30			
PH	Mean ± SD	7.38 ± 0.03	7.25 ± 0.05	10.068	0.000	HS
	Range	7.32 – 7.44	7.13 – 7.33			
PaCO2	Mean ± SD	37.40 ± 3.80	26.96 ± 4.71	8.268	0.000	HS
	Range	32 – 44	19 – 36			
PaO2	Mean ± SD	91.65 ± 1.90	76.63 ± 10.01	6.609	0.000	HS
	Range	89 – 95	52 – 90			
HCO3	Mean ± SD	21.65 ± 2.35	12.04 ± 2.56	13.441	0.000	HS
	Range	18 – 26	8 – 18			

•: Independent-test; *: Chi-square test; ǂ: Mann-Whitney test
NS: Non significant; S: Significant; HS: Highly significant

It shows that there was highly statistically significant decrease in parameters of ABG (PH, PCO2, PAO2, and HCO3) in the diseased group compared to the control group.

Table (4): Comparison between the two studied groups regarding the investigations of all cases

		Control group	Cases group	Test value	P-value	Sig.
		No.= 20	No.= 30			
UREA (mg/dl)	Mean ± SD	24.25 ± 7.70	54.03 ± 29.20	-4.445•	0.000	HS
	Range	12 – 40	10 – 120			
Creatinine (mg/dl)	Mean ± SD	1.14 ± 0.27	1.76 ± 0.99	-2.725•	0.009	HS
	Range	0.5 – 1.6	0.9 – 5.7			
RBS (mg/dl)	Mean ± SD	72.15 ± 11.17	79.00 ± 29.65	0.985•	0.330	NS
	Range	55 – 90	45 – 186			
CRP	Median (IQR)	6 (3 – 6)	24 (6 – 36)	-4.657ǂ	0.000	HS

(mg/l)	Range	3 – 6	3 – 168			
Na (meq/dl)	Mean ± SD	138.45 ± 4.12	132.33 ± 7.32	3.390•	0.001	HS
	Range	132 – 145	122 – 150			
K (meq/dl)	Mean ± SD	4.00 ± 0.46	4.32 ± 1.13	-1.184•	0.242	NS
	Range	3.4 – 5	3 – 7.2			
ALT (IU/l)	Mean ± SD	29.40 ± 4.97	61.83 ± 35.32	-4.066•	0.000	HS
	Range	20 – 39	10 – 125			
AST (IU/l)	Mean ± SD	21.45 ± 3.24	45.63 ± 26.27	-4.082•	0.000	HS
	Range	16 – 29	16 – 142			

•: Independent-test; *: Chi-square test; ǂ: Mann-Whitney test
NS: Non significant; S: Significant; HS: Highly significant

It shows that there was highly statistically significant difference regarding urea, creatinine, CRP, serum sodium and liver function between the two studied groups and no statistically significant difference between the diseased group and the controller regarding RBS and potassium.

Table (5): Comparison between the two studied groups regarding serum glial fibrillary acidic protein at the first 24 hours age

		Control group	Cases group	Test value•	P-value	Sig.
		No.= 20	No.= 30			
GFAP At 24hours age	Mean ± SD	0.53 ± 0.08	1.14 ± 1.25	-2.172	0.034	S
	Range	0.5 – 0.76	0.65 – 7.5			

•: Independent-test; *: Chi-square test; ǂ: Mann-Whitney test
NS: Non significant; S: Significant; HS: Highly significant

It shows that there was statistically significant difference regarding GFAP at 24hours age between the two studied groups.

DISCUSSION

Hypoxic-ischemic (HI) brain injury is one of the main causes of disabilities in term-born infant ,but due to difficulties regarding diagnosis and treatment of HI injury, there is an increasing need to find accurate method to diagnosis.

Although many potential biomarkers of brain damage exist, serum glial fibrillary acidic protein (GFAP) hold significant promise

in this population (Day and Thompson, 2009).

Our study showed no significant difference between the two studied groups regarding age of the mothers and parity with P value (0.457-0.400) respectively and this agree with (Chalak et al. (2014) who found no relation between maternal variable like age and parity and HIE.

In hypoxic ischemic encephalopathy at delivery the presence of meconium stained amniotic fluid indicates that fetal distress may have occurred and the affected infants may be depressed and fail to breathe spontaneously with low Apgar scores, neurological dysfunction in form of neonatal encephalopathy which may present as subnormal or depressed level of consciousness respiratory depression, abnormal muscle tone and strength, seizures activity may occur (Douglas and Weiss, 2015).

This study showed regarding the diseased group that 37% of In our study there was highly statistically significant difference in arterial blood gas finding done immediately after birth (PH-PaCO2-PaO2-HCO3) between the diseased group and the controller and this results agree with (Hesham et al. (2005) who found the same results in study done (20 diseased and 15 control) neonates also in agreement with(Brankica et al. (2012) who reported the same results.

In our study there was statistically significant difference regarding urea and creatinin between the two studied groups. This results in agreement with Hankins et al. (2002)).

In our study we found highly statistically significant difference between the two studied groups regarding liver function with (P=0.000).

But there was no statistically difference regarding blood glucose level with (P=0.330)between the diseased and the controller, this results agree with(Serdar et al. (2014) who reported the same results.

In our study there was statistically significant difference between the two studied groups regarding serum Na with (P=0.001).

These results agree With (Basu et al. (2010) who showed in their study significant hyponatremia ($p < 0.001$). also Gupta et al. (2005).

Glial Fibrillary Acidic Protein (G-FAP)is a monomeric filament protein localized predominantly in astroglial cells and released as a

consequence of brain damage and progressively increases according to the postmenstrual age in both term and preterm neonates (Okonkwo et al., 2013).

In our study there was statistically significant difference between the level of GFAP at24 hours age and the GFAP in the controller (Table 5) (P= 0.040),there this results agree with (Douglas et al. (2014) who reported that there was statistically significant difference regarding GFAP at24 hours of age in study done on (16 diseased and 11 control) neonates, also (Massaro et al. (2013) have recently reported optimal time of detection GFAP at 24 hours of life in study done on 27neonates with HIE.

CONCLUSION

- Levels of glial fibrillary acidic protein (GFAP) are higher in encephalopathic neonates than neurologically normal ones.
- Serial increase in level GFAP from birth in HIE neonates.
- The severity of the hypoxic-ischemic injury can be stratified at birth and postnatal because elevated GFAP in serum correlated with severity of HIE

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دراسه معدل البروتين الحمضي في الدم مصحوبا لدي الاطفال حديثي الولاده المصابين بالاعتلال الدماغي نتيجه نقص الاكسجين.

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الهدف من البحث: الاعتلال الدماغي لدي الاطفال حديثي الولاده من الامراض الغير متجانسه ويعرف من وجهه النظر العمليه باضطراب في الوظائف العصبيه في الايام الاولي بعد الولاده للاطفال الذين يولدون في الاسبوع الخامس والثلاثين من العمر الرحمي او بعد ذلك ويصاحب باختلال في الوعي وتشنجات وصعوبه في بدايه وتنظيم وظائف التنفس وهبوط في ردود الافعال.

وبالرغم من وجود العديد من المؤشرات الحيويه المحتمله لتلف الدماغ ولكن يعتبر البروتين الحمضي الخاص بالمخ والذي لا يفرز خارج الجهاز العصبي من الامال الواعده في تشخيص هذا المرض خاصه وانه يفرز عند اصابه المخ والحاجز الدماغي الدموي بالتلف نتيجه نقص الاكسجين.

تصميم البحث: تم البحث على 30 من الاطفال الحديثي الولاده المصابين بالاعتلال الدماغي نتيجه نقص الاكسجين الموجودين فى مستشفى باب الشعريه القاهره والمقسمين تبعا للتقسيمه سرنات ومقارنتهم 20 طفل حديثي الولاده أصحاء في نفس العمر والجنس.

الحالات المتضمنه في البحث:

الاطفال الحديثي الولاده المصابين بالاعتلال الدم نتيجه نقص الاكسجين.

الحالات المستبعده من البحث:

- حالات اختلال في وظائف التمثيل الغذائي.
- الحالات التى تعانى من اضرابات في الكروموسومات.
- حالات التشوهات الخلقية الكبرى.
- حالات التشوهات الفيروسيه الخلقية.

خضعت جميع الحالات سواء المرضيه أو الطبيعيه للاتي:

- أخذ التاريخ المرضى بالكامل سواء من الام.
- خضع كل المرضى للفحص الاكلينيكى الكامل.
- التحاليل الروتينيه شامله (صورة دم كاملة-بروتين تفاعلى سى-نسبة يوريا وكرياتنين-وصوديوم وبوتاسيوم-وسكر عشوائي –وغازات بالدم) والأبحاث المتخصصة الخاصة بقياس نسبه البروتين الحمضي بالدم.

نتائج البحث: تبين ان معدل البروتين الحمضي يزاد نسبته ف الدم لدي الاطفال حديثي الولاده المصابين بالاعتلال الدماغي نتيجه نقص الاكسجين. كما ان نسبته تتفاوت حسب درجه الاعتلال الدماغى.

الاستنتاجات: تبين من البحث ان معدل زياده البروتين الحمضى يزداد فى الاطفال اللذين تعرضو لنقص الاكسجين فى الدم وان حده تاثير نقص الاكسجين فى الدم على اجهزه الجسم يتناسب طرديا مع زياده معدل البروتين الحمضى فى الدم.

التوصيات: نوصى بقياس معدل البروتين الحمضى فى الدم للاطفال الذين تعرضوا لنقص الاكسجين فى الدم وذلك لمدى حده وتاثيره على اجهزه الجسم.