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Neuroglobin as a Neuroprotective in Neonates with Hypoxic Ischemic Encephalopathy

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Abstract

Background

Neuroglobin is a member of the globin family that reversibly binds oxygen and increases oxygen delivery to brain. It also protects brain in hypoxemic or state levels, and so, decreases brain insult. We aimed to evaluate whether neuroglobin can be considered a neuroprotective in neonates with hypoxic-ischemic encephalopathy (HIE) by measuring its serum level in such cases.

Materials and Methods: This is a prospective case – control study that was conducted in Minia University Children's hospital, El-Minya, Egypt on 30 term neonates who were diagnosed to have hypoxic–ischemic encephalopathy and another 30 apparently healthy term neonates as a control group. For both cases and controls, detailed history, clinical examination and serum neuroglobin level were done, while arterial blood gases, serum electrolytes, liver function, renal function tests, CBC, CRP, and CT- brain were done for cases only.

Results: The results showed that serum neuroglobin levels were significantly higher in cases with hypoxic-ischemic encephalopathy than control group ($p < 0.001$). In this study, serum neuroglobin levels were higher ($p < 0.001$). We observed a weak negative correlation between serum neuroglobin level and Apgar score at one minute in studied cases with HIE. Also, we found that presence of both brain edema and hemorrhage in CT brain in cases with HIE was associated with a high mean serum neuroglobin level, than in either finding alone ($p < 0.001$).

Conclusion

Neuroglobin could be considered as a neuroprotective in neonatal cases with hypoxic ischemic encephalopathy and this may be considered in the future potential therapeutic options in such cases.

Key Words: Hypoxic Ischemic Encephalopathy, Egypt, Neonates, Neuroglobin.

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1- INTRODUCTION

Hypoxic- Ischemic Encephalopathy (HIE) is a type of neonatal encephalopathy caused by systemic hypoxemia and/or reduced cerebral blood flow resulting from an acute antepartum or intra partum event. Reduction of blood flow or gas exchange during the peripartum period can result in profound neurologic sequelae (1, 2). HIE can induce significant insult and long term irreversible morbid status (3). Hypothermia is now established as the standard treatment; efficacy is limited and has a therapeutic effect of 6 hours (4). Neuroglobin (expressed in the nervous system) is an oxygen-carrying globin (5). Neuroglobin has has a function similarity to that of myoglobin and could act as oxygen transporter to neuronal mitochondria (6). Central and peripheral nervous systems express neuroglobin in neurons and their highest levels are found in the hypothalamus up to 100-fold higher compared to the cerebral cortex, cerebellum, and hippocampus, confirmed at both the transcript and protein levels (7, 8). Also, high neuroglobin levels were found in nonneural high metabolically active tissues, like the retina and several endocrine tissues (9-11). It has been proved that neuroglobin is closely related to brain damage of hypoxemic causes (12). Various pathological conditions up regulate neuroglobin expression when responding to ischemic or hypoxemic challenge (13). The aim of this study was to evaluate serum neuroglobin level in neonates with hypoxic ischemic encephalopathy, to estimate if it could be considered as a neuro protective agent in such cases or not.

2- MATERIALS AND METHODS

2-1. Study design and population

This case-control study was conducted on 30 neonates diagnosed to have HIE according to American Academy of Pediatrics (AAP) criteria (14) for diagnosis of HIE, that were admitted in Neonatal Intensive Care Unit, Minia University Children's Hospital in El Minya, Egypt during the period from May 2019 to December 2019. Another 30 apparently healthy term neonates with cross matched age and sex were chosen as a control group.

2-2. Methods

Both study and control groups were subjected to the following:

1- Detailed history taking regarding:

- Gestational age
- Gender
- Mode of delivery and
- Apgar score at one and five minutes of age.

2- General examination, with assessment of gestational age using Ballard score.

3- Thorough neurological examination with assessment of severity of hypoxic ischemic encephalopathy by using Sarnat and Sarnat staging (1976) (15).

4-Investigations in the study group only:

- Umbilical cord arterial blood gases
- Serum urea, creatinine, Liver function tests.
- Serum electrolytes
- d -CBC, CRP and
- CT- brain.

5-Serum neuroglobin level by ELIZA in both study and control groups.

2-3. Inclusion and exclusion criteria

Inclusion criteria include: full term neonates, fulfilling the diagnosis of HIE according to AAP criteria, persistently low Apgar score (0-3 for longer than 5 minutes), presence of neurological signs, e.g. seizures, coma; multiple organ involvement, base deficit more than 10. Exclusion criteria were: Preterm neonates delivered before 37 weeks' gestation, newborns with major congenital anomalies, and newborns with strong suspicion of sepsis.

2-4. Ethical consideration

The study was conducted according to the principle of Helsinki and was revised and approved by the Faculty of Medicine, Minia University Ethical Committee. Informed written consents from participant parents were obtained.

2-5. Data Analyses

The collected data were coded, tabulated and statistically analyzed using SPSS program (Statistical Package for the Social Sciences) software, version 20. Descriptive statistics were done for numerical data by mean, standard deviation. For parametric quantitative data: independent sample test was used. For quantitative data: chi square test was used. Correlation between two quantitative variables was done by using Pearson's correlation coefficient and for qualitative variables by using Spearman's correlation coefficient. The level of significance was taken at p <0.05.

3- RESULTS

Demographic and clinical data of the study and control groups are represented in **Table.1**. Where, in the study group, Apgar score at one (3.96±0.73), and five minutes (6.52±0.58) was significantly lower than the control group ($p < 0.001$), also cesarean section delivery (36.7%) rate was significantly higher in the study group, than the control group (6.7%) ($p < 0.001$). Regarding the severity of hypoxia in the studied cases, 25% of patients were grade I, 35 % were grade II, while 40% were grade III as shown in **Figure.1**. Serum neuroglobin level (ug/L) was significantly higher in cases with HIE, than in the control group (69.79±17.08 and 23.49±6.54, respectively) ($p < 0.001$). The higher the grade of HIE, the higher the neuroglobin level, as shown in **Table.2**.

Table-1: Demographic and clinical data of both study and control groups, n=60.

Variables	Cases, n =30	Control, n = 30	*P* -value
Gestational age Range Mean ± SD	 37-39 37.52±0.71	 37-39 38.16±0.89	 0.80
Gender Male Female	 16(53.3%) 14(46.7%)	 16(53.3%) 14(46.7%)	 --
Mode of delivery Vaginal delivery Cesarean section	 19(63.3%) 11(36.7%)	 28(93.3% 2(6.7%)	 0.001
Apgar score at 1 minute Range Mean ±SD	 3-5 3.96±0.73	 8-10 9.2±63	 <0.001
Apgar score at 5 minutes Range Mean± SD	 5-7 6.52±0.58	 9-10 9.52±0.12	 <0.001

SD: Standard deviation.

Table-2: Comparison between HIE cases and control group regarding serum neuroglobin level.

Parameter	Cases, n=30	Control, n=30	*P*-value
Serum neuroglobin level(ug/L) Range Mean ±SD	 46.5-108.3 69.79±17.08	 15.1-39.1 23.49±6.54	 <0.001

SD: Standard deviation, HIE: Hypoxic- Ischemic Encephalopathy.

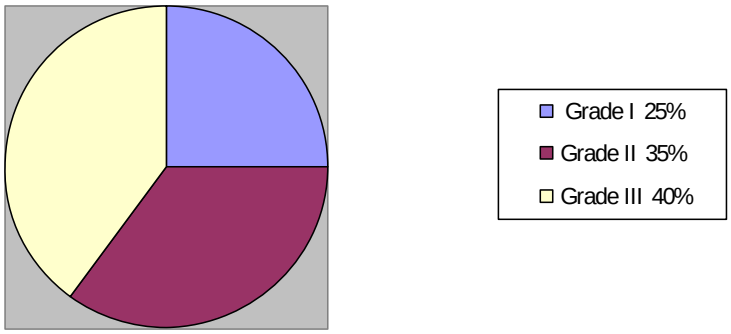

Fig.1: Classification of HIE cases according to severity of hypoxia.

HIE: Hypoxic- Ischemic Encephalopathy.

No significant correlation between serum neuroglobin level and gestational age and Apgar score at 1 and 5 minutes, was observed, **Table.3**. Mean serum neuroglobin level was higher in HIE cases with grade III hypoxia than other grades (p= 0.04), **Table.4**.

Table-3: Correlation between serum neuroglobin levels, gestational age, Apgar score and serum electrolytes in the studied group with HIE.

Parameter	Serum neuroglobin level (ug/L)	
	Pearson's correlation	*P* -value
Gestational age	0.063	0.765
Apgar score at 1 minute	-0.182	0.385
Apgar score at 5 minutes	0.015	0.945
Serum Na	-0.450	0. 24
Serum K	0.263	0.194
Serum Ca	0.192	0.357

Ca: Calcium, K: Potassium, Na: Sodium, HIE: Hypoxic- Ischemic Encephalopathy.

Table-4: Comparison between the different grades of hypoxia in HIE cases regarding serum neuroglobin level.

Grade of hypoxia	Median serum neuroglobin level (ug/l)	P-value
I	46.23	0.04*
II	62.14	
III	82.80	

*: Significant.

Mean serum neuroglobin level was higher in cases presented with convulsions (p=0.035), other than neurological findings in the study group as shown in **Figure.2**. Presence of both brain edema and hemorrhage in CT brain in cases with HIE, was associated with a high mean serum neuroglobin level than in each finding (p = 0.04) **Table.5**.

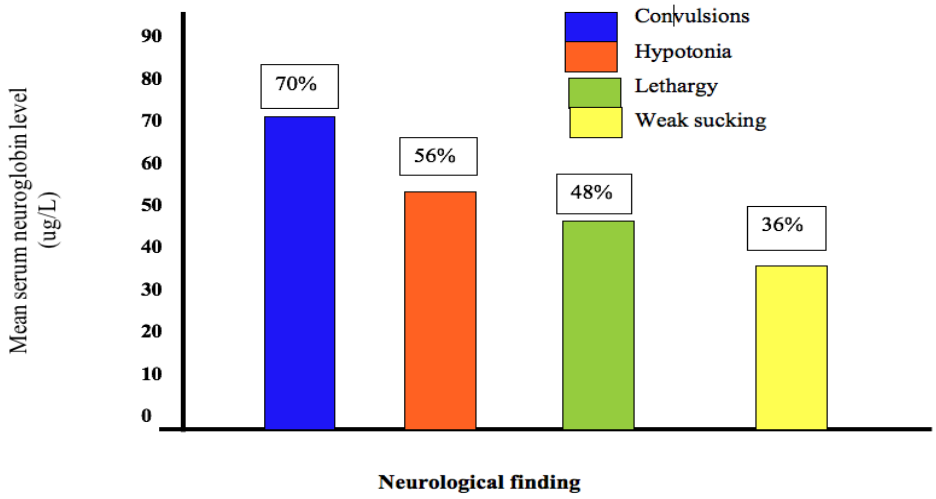

Fig.2: Correlation between mean serum neuroglobin level and neurological findings in the studied group with HIE. HIE: Hypoxic- Ischemic Encephalopathy.

Table-5: Mean serum neuroglobin level and CT brain finding in the studied group with HIE.

Parameter	(I) Edema, n=19	(II) Hemorrhage, n=7	(III) Both, n=4	P- value		
Serum Neuroglobin level				0.044		
				I vs. II	I vs. III	II vs. III
Range	46.5-93	52.4-68.4	89.1-108.3	0.555	0.025*	0.02*
Mean ±SD	68.18±16.22	60.76±6.48	96.2±10.53			

Kruskal Wallis test for non-parametric quantitative data between the three groups followed by Mann Whitney test between each two groups. *: Significant. SD: Standard deviation.

4- DISCUSSION

The aim of this study was to evaluate serum neuroglobin level in neonates with hypoxic ischemic encephalopathy, to estimate whether it could be considered as a neuro protective agent in such cases or not. In cases with hypoxic-ischemic encephalopathy, serum neuroglobin levels were significantly higher compared to control group and were higher in hypoxic cases presented with seizures more than those with other neurological deficits. Despite the improvements in perinatal care, perinatal HIE has been shown to adverse outcome worldwide, (16). Neuroglobin (NGB), is a novel member of the heme–binding globin family (16-20). NGB is an endogenous molecule exhibiting neuro protective role against hypoxic/ischemic injuries (21-23). In this study, we evaluated serum level of NGB in term neonates with HIE. (24). A higher prevalence of HIE in male gender was observed in other studies (25, 26). Females have higher catecholamines than males which is inconsistent with our results. Concerning mode of delivery in our studied cases with HIE, 10 out of 30 cases (33.3%) were delivered by cesarean section while the remaining 20 (66.7%) were delivered vaginally. Babies born by cesarean section were more prone to HIE than those delivered vaginally (27) which does not agree with our results. A larger sample size is needed to confirm these findings. Serum neuroglobin level was significantly higher in HIE cases than controls ($p<0.001$) matching the results of many previous research studies (22, 28, 29). Neuroglobin over expression was found to have a neuroprotective effect

during hypoxic – ischemic injury. Neuroglobin level increases in oxidative stress- related insults (20), and it is postulated that neuroglobin is seizure and ocular hypertension protective. Neuroglobin is up-regulated in most ischemic conditions regardless of the cause (30). The underlying mechanisms, however, are not fully clarified. It seems to possess a protective role in the brain only after up-regulation (31). Neuroglobin also has anti apoptotic properties acting on mitochondrial and cytosol mechanism of pathology. It can be considered as a potential target to decrease neural damage, and its enhanced expression post brain injury probably reflects endogenous mechanisms of neuro protection (31). Our study showed that median serum neuroglobin levels were higher in HIE cases presented with grade III hypoxia than other grades, in addition, it was also observed that its levels were higher in HIE cases with CT-brain findings of both edema and hemorrhage than either finding alone. The possible explanation for these findings is that the expression of neuroglobin in serum could be directly related to the degree of the hypoxic – ischemic brain insult.

4-1. Study Limitations

Lower number of cases was the main limitation of our study in addition to financial challenges that interfere with more kits for neuroglobin as saying.

5- CONCLUSION

We could conclude that neuroglobin is increased in neonates with HIE and that could imply its role as a neuro protective in hypoxic /ischemic insults and it could be used as a future potential therapeutic approach in treatment of HIE in neonates. We recommend conducting a study on a greater number of cases to validate this point and to understand the exact role of neuroglobin in cases of HIE in neonates.

6- CONFLICT OF INTEREST: None.

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