Serum Neuron-Specific Enolase (NSE) as Prognostic Biomarker in Neonatal Encephalopathy

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Abstract

Introduction: Neuron-specific enolase (NSE) is considered a valid marker in neonatal encephalopathy. Our study aims to evaluate the value of NSE in the early detection and prognosis of neonatal encephalopathy with different etiologies. **Subjects and methods:** A prospective case control study was conducted in the NICU of Suez Canal University Hospital. The study population was divided into two groups, case group (n=24): neonates recently diagnosed with encephalopathy, and control group (n=24): full-term healthy neonates who match age and gender. Full medical history, physical examination, and laboratory investigation including NSE serum level. Six-month follow-up was maintained to assess the neurodevelopment outcomes. **Results:** Our study revealed that the case group showed a significantly higher level (P value <0.001) of serum NSE. HIE showed significantly the highest frequent etiology of NE. Sever NE showed a 25% mortality rate during admission and 66.7% in 6-month follow-up. Serum NSE at a cut point-off level of 17.35 showed 95.8% sensitivity and 29.2% specificity for the diagnosis of NE with a p-value <0.001. **Conclusions:** Serum NSE is a reliable marker for the diagnosis of different stages of NE caused by different etiologies. With moderately low predictive value for long-term outcomes in NE infants.

Keywords: Neuron-specific enolase - neonatal encephalopathy

Introduction:

"Encephalopathy denotes a pathological condition characterized by cerebral tissue dysfunction or damage, clinically manifesting as alterations in mental status. **(Volpe 2012)**. In neonates, the assessment of altered mental status is particularly complex due to the absence of well-defined, objective markers for quantifying consciousness. The Sarnat staging system, which stratifies clinical symptomatology into mild, moderate, and severe categories, remains the predominant clinical classification framework for neonatal encephalopathy **(Sell et al. 2017)**.

Neonatal encephalopathy, while most frequently attributed to acute hypoxic-ischemic insults, exhibits a heterogeneous etiology. The preponderance of research focusing on hypoxia-ischemia stems from the fact that alternative causative factors are often delineated as discrete diagnostic entities, encompassing infectious pathologies (e.g., meningitis), inborn errors of metabolism (e.g., urea cycle defects), and other specialized diagnoses. Consequently, the onset of neonatal encephalopathy can occur across a spectrum of developmental periods, including the prenatal, perinatal, and immediate postnatal phases. These varied etiologies are primarily rooted in metabolic, genetic, infectious, and traumatic insults. Irrespective of the specific cause, the unifying pathophysiological mechanism underlying neonatal encephalopathy is the disruption of homeostatic equilibrium, culminating in cerebral tissue damage, functional neurological aberrations, and structural brain alterations **(Sell et al. 2017)**.

Neuroimaging modalities, such as magnetic resonance imaging (MRI), and neurophysiological assessments, including electroencephalography (EEG) and evoked potentials, serve primarily for the evaluation of disease severity and prognostic determination in neonatal encephalopathy, rather than for primary diagnosis. Notably, a significant proportion (15-30%) of neonates classified with mild encephalopathy according to the Sarnat staging system may exhibit unremarkable MRI findings **(Bonifacio and Hutson 2021)**. This underscores the clinical imperative for the identification of an early, sensitive, and specific biomarker for the accurate and timely diagnosis of neonatal encephalopathy.

Neuron-specific enolase (NSE), a cell-specific isoenzyme of the glycolytic enzyme enolase, serves as a valuable biomarker for the assessment of neural cell integrity. Its utility is predicated on its manifestation as a delayed indicator of neuronal injury. NSE demonstrates high specificity for alterations within neuronal populations and peripheral neuroendocrine cells **(Isgrò, Bottoni, and Scatena 2015)**.

Our study aim to evaluate the value of NSE in early detection and prognosis of neonatal encephalopathy with different etiology.

Patients and Methods

Ethical consideration:

1. Our study was approved by the ethical committee of Faculty of Medicine, Suez Canal University.
2. An informed consent was obtained from all caregivers of participating children.
3. The results and data of the study are confidential, and the patient has the right to keep it.
4. the cargiver has the right to withdraw from the study at any time
5. The authors received no financial support for the research, authorship, and/or publication of this article.
6. No conflict of interest regarding study or publications

Sample size calculations:

The calculated sample size is 45, it was calculated using OpenEpi tool, Version 3, open-source calculator--SSPropor (OpenEpi - Toolkit Shell for Developing New Applications). Prevalence/proportion of the neonatal encephalopathy **(P)** = 0.003 **(Kurinczuk, White-Koning, and Badawi 2010)**. The critical value that divides the central 95% of the Z distribution from the 5% in the tail **($Z_{\alpha/2}$)** = 1.96. The margin of error/Width of confidence interval **(E)** = 0.05,

Sample size (n) = $(Z_{\alpha/2} /E)^2 * P(1-P)$

Inclusion Criteria:

1. A full term neonate who recently (within 48h) diagnosed with encephalopathy due to different etiologies (ex: HIE, perinatal infection, placental abnormalities, coagulation disorders, inborn error, vascular stroke and idiopathic causes)
2. Neonate age ≤ 28 days of age

Exclusion Criteria:

1. Maternal drug use of narcotics (as it affects the mental status of the neonate)

Method

The study will include 2 groups: Control group (n=24): full term health neonates without encephalopathy or any neurological disease who will come for follow up in outpatient clinic of pediatric at Suez Canal University Hospital and the study group (n=24): neonates recently diagnosed with encephalopathy.

All neonates were subjected to:

Full history taking:

This included gender, gestational age, mode of delivery, risk factors of infection (as Premature rupture of membranes (PROM) or intrapartum hemorrhage), maternal diseases (as diabetes mellitus (D.M) or hypertension (HTN)) and drug history, post-natal history (resuscitation, hypoxic event, delay of first crying or cyanosis), family history of similar condition

Physical examination:

a) Congenital anomalies
b) Encephalopathy stage according to Modified Sarnat Staging for Neonatal Encephalopathy (Table 1) **(Power et al. 2019)**.
c) Anthropometric assessment

Laboratory investigation

a) Neuron-specific enolase (NSE) level by enzyme immunometric assay utilizing enzyme immunometric assay Kit.
b) Routinely laboratory investigation which done for the neonate previously (ex: CBC, ABG, LFT, KFT, CRP, blood culture, CSF analysis, coagulation profile and metabolic screening as ammonia and lactate) for the study group only.
c) Routinely imaging investigation which done for the neonate previously (ex: cranial ultrasound, CT brain and Brain MRI) for the study group only.

Table 1: The Modified Sarnat Staging for Neonatal Encephalopathy **(Power et al. 2019)**

Severity		Stage 1 (mild)	Stage 2 (Moderate)	Stage 3 (Severe)
Level of consciousness		Hyperalert	Lethargic / Obtunded	Stupor or coma
Activity		Normal	Decreased	Absent
Neuromuscular Control	Muscle tone	Normal	Mild hypotonia/hypertonia	Flaccid/rigid
	Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
	Tendon reflexes	Overactive	Overactive	Decreased or Absent
Complex reflexes	Suck	Weak	Weak/absent	Absent
	Moro	Strong, low threshold	Weak, incomplete, high threshold	Absent
	Tonic neck	Slight	Strong	Weak or absent
Autonomic Nervous System	Pupils	Dilated pupil	Constricted pupil	Variable: often unequal, poor light reflex, fixed, dilated
	Heart rate	Tachycardia	Bradycardia	Variable
	Respiratory rate	Regular	Periodic breathing	Apnoea
Seizure		None	Common; focal or multifocal	Uncommon (excluding decerebration)

The collection of serum Samples From participants is as follows:

Three ml whole blood was obtained on a plain tube and then centrifuged after (20) minutes at 2500 rpm for 10 minutes to collect serum in separate Eppendorf. Then, the serum was frozen at -20°C then thawed to carry out the NSE assays. NSE was measured using enzyme-linked immunosorbent assay (ELISA), a sandwich method using Human Neuron-specific enolase (NSE), Shanghai, China Cat.No : E0937Hu). micro-ELISA plate has been pre-coated with an antibody specific to Human NSE. Both standard samples and participant samples were added to the micro-ELISA plate wells. Samples were added to the wells as follows; 40µl serum, then both NSE-antibody 10µl and Streptavidin-HRP 50µl and incubated 60 min at 37°C then washed five times. Then, 50µl chromogen A and 50µl chromogen B were added and incubated (10) min at 37°C away from light. Finally, 50µL stop solution was added to each well. Then the optical density (OD) was estimated within 10 minutes using a spectrophotometer at 450 nm wavelength. The OD is proportional to the concentration of Human NSE. A standard curve was plotted on linear-linear graph paper, with standard concentration on the x-axis and OD values on the y-axis.

A six-month follow-up for the study population was maintained to assess the general physical status and the neurodevelopment of the individual.

Results:

Our results will be demonstrated in the following tables and figures

Table 2: Sociodemographic data of the study population

Variable		Case Group (n=24)	Control Group (n=24)	P value
Gestational age		38.6 ± 1.54	38.1 ± 1.96	0.51
Gender	Male	12 (50%)	11 (45.8%)	0.77
	Female	12 (50%)	13 (54.2%)	
Mode of delivery	NVD	9 (37.5%)	11 (45.8%)	0.56
	CS	15 (62.5%)	13 (54.2%)	

Table 2 shows the sociodemographic data of the study groups. There was no statistical difference among the different groups according to gestational age, gender and mode of delivery

Table 3: Baseline assessment of the neonates in the study population

Variable	Case Group (n=24)	Control Group (n=24)	P value
Birth weight (gm)	2952.1 ± 427.1	3110.5 ± 413.1	0.43
Apgar score at birth	4.5 ± 1.4	6.8 ± 0.9	0.34

Apgar score at 5 min	6.1 ± 1.5	8.6 ± 1.1	0.15

Table 3 demonstrated comparable birth weights and Apgar scores (at 1 and 5 minutes) between the case and control groups.

Table 4: Lab results of the study population

Variables	Case Group (n=24)	Control Group (n=24)	P value
Serum NSE	44.7 ± 16.4	14.9 ± 4.6	<0.001
pH at diagnosis	7.11 ± 0.20	7.39 ± 0.17	0.002

laboratory analysis, as detailed in Table 4, revealed a statistically significant elevation ($P < 0.001$) in serum neuron-specific enolase (NSE) levels within the case group compared to the control group. Furthermore, the case group exhibited a statistically significant ($P = 0.002$) degree of acidosis, as evidenced by lower pH values, compared to the control group.

Table 5: Outcome among the NE stages

Outcome		Mild NE (n=8)	Moderate NE (n=12)	Severe NE (n=4)	P value
During admission	Dead	0 (0%)	1 (8.3%)	1 (25%)	0.002
	Neurological defect	0 (0%)	7 (58.3%)	3 (75%)	
	Anti convulsions	0 (0%)	1 (8.3%)	2 (50%)	0.02
6 months follow up	Dead	1 (12.5%)	1 (9.1%)	2 (66.7%)	0.03
	Neurological defect	0 (0%)	3 (27.3%)	1 (33.3%)	
	Anti convulsions	0 (0%)	4 (36.4%)	1 (33.3%)	0.01

Table 5 shows the outcome among the different NE stages. During admission, the stages showed significant differences regarding the outcome (P value= 0.002), as mild NE showed no dead neonates while there was only one dead neonate in each moderate NE and severe NE. Six months later the follow-up revealed one dead neonate in each mild NE (out of 8 survived neonates) and moderate NE (out of 11 survived neonates) while severe NE showed 2 dead neonates (out of 3 survived neonates).

Regarding the use of regular anti-convulsions to control the seizures, the stages showed significant differences (P value= 0.02) between the different stages. Regular anti-convulsion is needed only in 1 neonate (8.3%) with moderate NE and 2 neonates (50%) with severe NE during admission.

Table 6: Correlation between serum NSE level and the outcome during admission and after 6 months of follow-up

Variable		Correlation	P value
During admission	Death	-0.17	0.43
	Neurological defect	0.68	<0.001*
	Anti-convulsion	0.66	0.001*
6 month follow up	Death	-0.47	0.22
	Neurological defect	0.54	0.007*
	Anti-convulsion	0.45	0.027*

Table 6 shows the correlations between serum NSE levels and the outcome during admission and after 6 months of follow-up. Serum NSE showed a positive significant correlation with the incidence of neurological defect and the utilization of anti-convulsion medications during admission and after 6 months of follow-up.

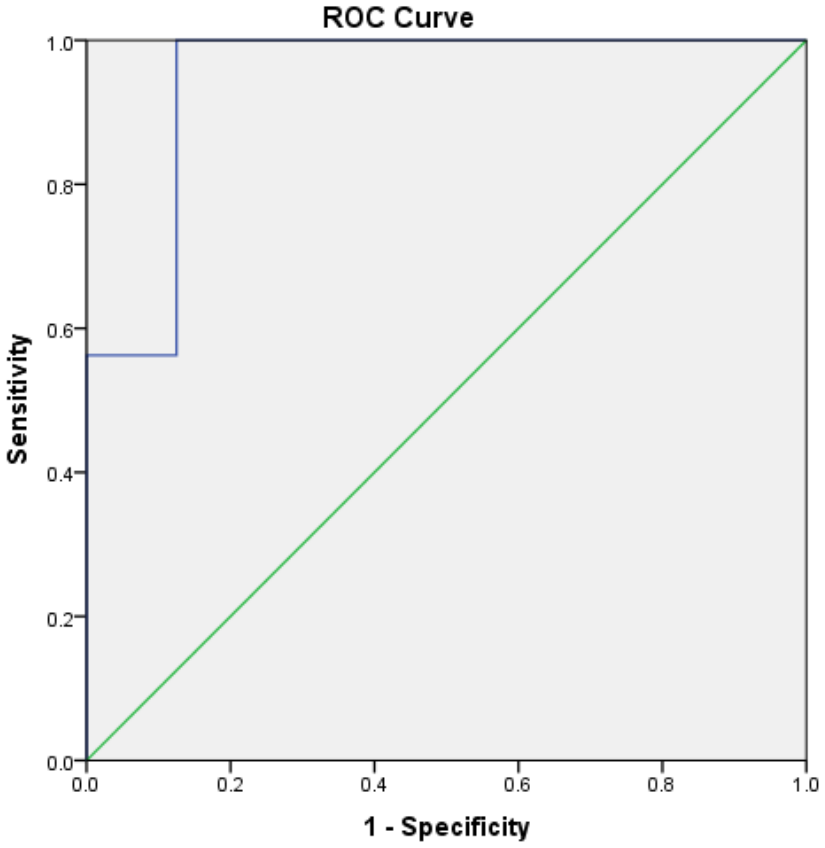

Figure 1: ROC curve of the relation between serum NSE level and classification of moderate to severe stages of NE

ROC curve of the relation between serum NSE level and moderate to severe stages of NE in Figure 1 shows that the cut point of 45.4, the area under the curve was 95% (95%CI; 84%-100%) with 93.8% sensitivity and 51% specificity. The p-value <0.001.

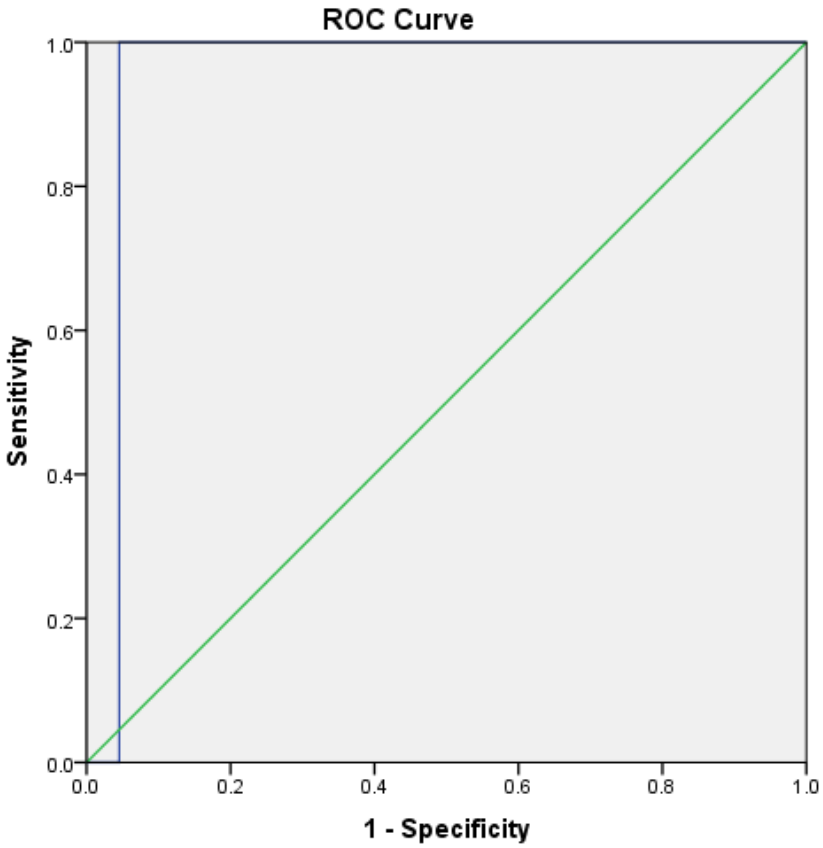

Figure 2: ROC curve of the relation between serum NSE level and death as an outcome

Figure 2 shows the ROC curve of the relation between serum NSE level and death as an outcome. At the cut point of 63.3, the area under the curve was 95.5% (95%CI; 86.8%-100%) with 50% sensitivity and 45% specificity. The p=value = 0.037.

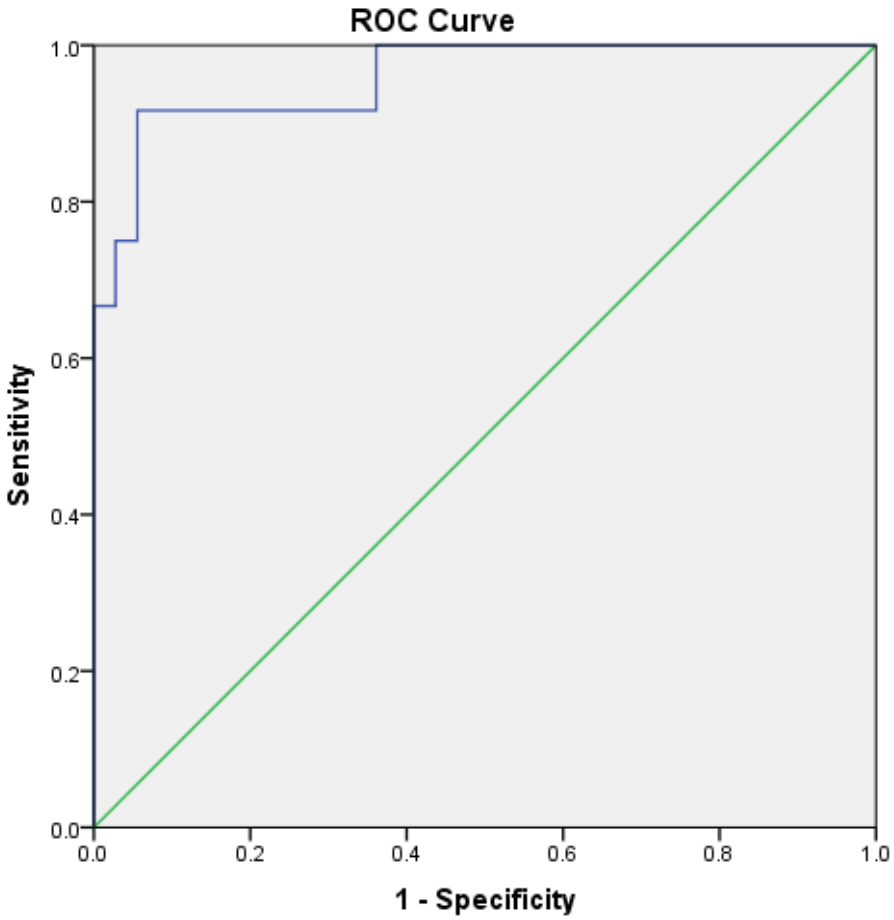

Figure 3: ROC curve of the relation between serum NSE level and neurological defect during admission as an outcome

Figure 3 shows the ROC curve of the relation between serum NSE level and neurological defect during admission as an outcome. At the cut point of 21.55, the area under the curve was 95.8% (95%CI; 89.6% -100%) with 91.7% sensitivity and 36.1% specificity. The p-value = 0.001.

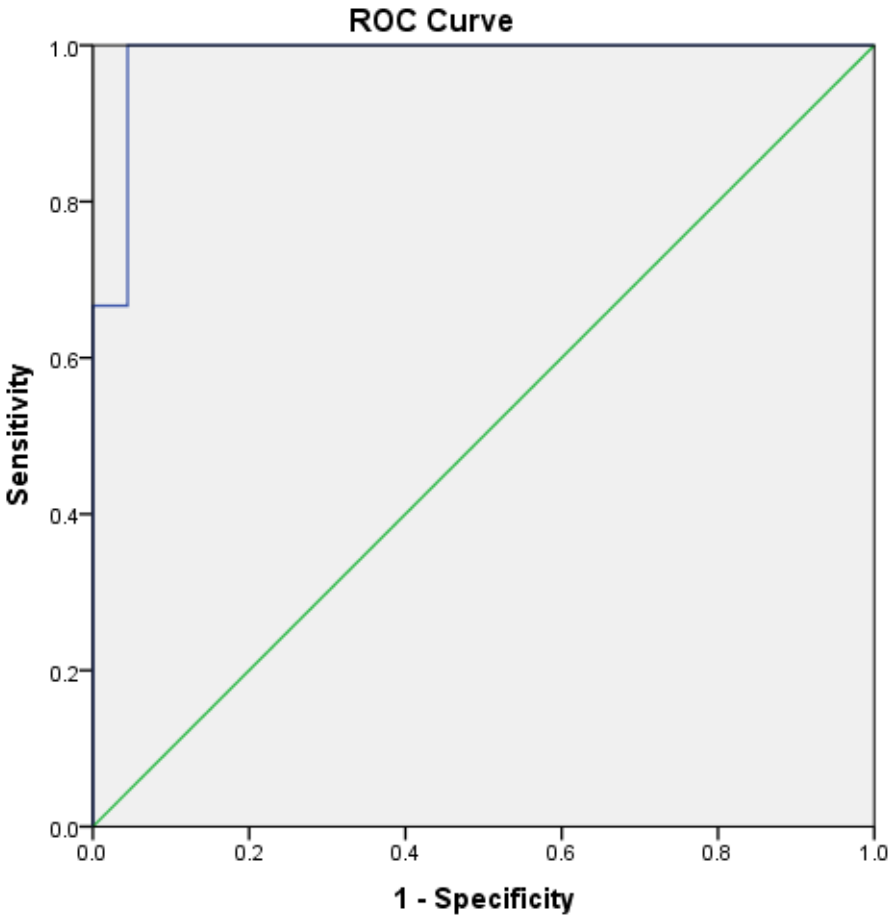

Figure 4: ROC curve of the relation between serum NSE level and neurological defect after 6 months follow up as an outcome.

Figure 4 shows the ROC curve of the relation between serum NSE level and neurological defect as an outcome after 6 months of follow-up. At the cut point of 59.3, the area under the curve was 98.5% (95%CI; 95% -100%) with 66.7% sensitivity and 4.4% specificity. The p value= 0.005.

Discussion

To evaluate cerebral insults and predict neurological prognosis in neonates, a multitude of diagnostic modalities have been employed in conjunction with clinical assessments. Over the past decade, research has focused on the identification and validation of diverse biochemical markers indicative of brain injury following neonatal encephalopathy (NE) **(Nagdyman et al. 2013)**,**(Infante et al. 2003)**.

This study demonstrated that hypoxic-ischemic encephalopathy (HIE) constituted the predominant etiology of neonatal encephalopathy (NE) within the case cohort, accounting for 62.5% of cases, surpassing other identified causes. This finding aligns with observations reported by Namusoke et al., which indicate HIE as the most frequent cause of NE, with reported incidences of 1.5 per 1000 live births in industrialized nations and a significantly broader range of 2.3 to 26.5 per 1000 live births in resource-limited settings. However, it is noteworthy that comprehensive epidemiological data regarding the prevalence and sequelae of NE remain deficient in numerous developing nations. **(Namusoke et al. 2018)**. This explains the high number of HIE neonates in this study.

The study demonstrated statistically significant elevation of serum NSE levels in neonates with encephalopathy (44.7 ± 16.4 mg/L) compared to healthy controls (14.9 ± 4.6 mg/L, $p < 0.001$). This elevation results from compromised blood-brain barrier integrity, leading to NSE release into systemic circulation and cerebrospinal fluid. These findings are consistent with previous investigations in HIE neonates **(Soliman AM and Abdel-Moety 2011; Seema et al. 2014; Panda et al. 2020)**.

A statistically significant inverse correlation (-0.92, $p = 0.002$) was demonstrated between serum NSE concentrations and 5-minute Apgar scores. Prolonged physiological deterioration beyond five minutes correlates with cerebral injury and concomitant NSE elevation, consistent with previous HIE investigations **(Nagdyman et al. 2012; Attia et al. 2016)**. This investigation yielded non-significant correlations between serum neuron-specific enolase (NSE) levels and various clinical parameters, including gestational age, birth weight, pH, and Apgar score at birth.

A statistically significant positive correlation ($r = 0.826$, $P < 0.001$) was observed between serum NSE concentrations and NE severity staging. This suggests serum NSE serves as a reliable biomarker for assessing encephalopathy severity, consistent with León-Lozano et al. (2020) findings showing significantly elevated NSE in severe HIE compared to mild and moderate cases **(Blennow et al. 2011; Falero et al. 2011)**.

The study documented 25% mortality among severe NE cases, with 50% of survivors exhibiting neurological sequelae at discharge. A statistically significant association was observed between serum NSE levels and diverse neurological deficits, reinforcing NSE's predictive capacity for assessing HIE severity and subsequent neurological sequelae **(Varsami et al. 2013)**. Conversely, independent investigations have documented a robust correlation between cerebrospinal fluid (CSF) neuron-specific enolase (NSE) concentrations and the severity of hypoxic-ischemic encephalopathy (HIE), the extent of cerebral injury, and the manifestation of resultant neurological deficits **(Blennow et al. 2011; Vasiljević et al. 2012)**. According to this study, serum NSE is a good prognostic marker for neurological defects in neonates with NE.

Serum NSE demonstrated 93.8% sensitivity and 51% specificity for predicting moderate or severe NE using a cut-off value of 45.4 mg/L. These findings are comparable to León-Lozano et al. (2020) results showing 81% sensitivity and 65% specificity at 50 mg/L cut-off **(Leon-Lozano et al. 2020)**, and Nagdyman et al. (2012) reporting 79% sensitivity and 70% specificity at 40.0 mg/L cut-off **(Nagdyman et al. 2012)**.

For predicting neonatal mortality, serum NSE showed 50% sensitivity and 45% specificity using a 63.3 mg/L cut-off. In contrast, cerebrospinal fluid NSE analysis by León-Lozano et al. (2020) demonstrated superior performance with 86% sensitivity and 98% specificity at 108 mg/L cut-off, attributed to higher CSF concentrations providing more direct cerebral injury reflection **(Leon-Lozano et al. 2020)**.

Limitation

Despite its prognostic value, serum NSE lacks discriminatory power in differentiating between various NE etiologies ($P = 0.213$), necessitating reliance on clinical presentation and supplementary investigations for differential diagnosis. Additionally, factors including gestational age, physiological stress, and non-neurological trauma may confound NSE levels, limiting its specificity during immediate postnatal periods.

Conclusion:

Serum NSE is considered to be a reliable marker for the diagnosis of different stages of NE caused by different etiologies. With moderately low predictive value for long-term poor outcomes in NE infants

Recommendation

- A large sample size with different etiology and stages of NE should be included
- Long period of follow up studies needed to better evaluation of the role of serum NSE in prediction of neurological defect and its type.
- Different markers should be used in assessing the severity and the morbidity of neonatal encephalopathy

Author distribution

- **Ahmed Meshref**: physical examination, data collection, manuscript writing
- **Hala Elhagrasy**: Supervision, data analysis.
- **Hebat-Allah Hassan Nashaat**: lab analysis and results interpretation.
- **Marwa Ahmed Mohamed**: methodology, critical revision.

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